Evaluation of Various Organic Fertilizer Substrates and Hydraulic Retention Times for Enhancing Anaerobic Degradation of Explosives-Contaminated Groundwater While Using Constructed Wetlands at the Milan Army Ammunition Plant, Milan, Tennessee

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This document describes studies conducted at the Milan Army Ammunition Plant (MAAP) to improve the design, operation, and cost of gravel-based anaerobic cells when phytoremediating explosives-contaminated groundwater. A typical gravel-based wetland consists of an anaerobic cell for removing the bulk of the explosive-contaminates; and an aerobic cell for removing CBOD-5, nutrients, total suspended solids, and small quantities of explosive by-products. The cells are connected in series with the anaerobic cell being the first cell.

To conduct this study, small-scale anaerobic test cells were used to determine: 1.) If the hydraulic retention time of a large demonstration-scale anaerobic cell at MAAP could be reduced and 2.) if other carbon sources could be used as an anaerobic feedstock. The study results indicated that:

- **The existing anaerobic cell's 7.5-day retention time should not be reduced since residual explosive by-products were present in the effluent of treatments with a 3.5-day retention time.**
- **Daily application of a relatively soluble substrate, such as molasses syrup, will provide better explosives removal than periodic application of less soluble substrates like milk replacement starter and sewage sludge.**
- **Molasses syrup could be, and should be, used as a substitute for milk replacement power.**

The recommendation to use molasses syrup was based on: 1.) The lower cost of molasses syrup as compared to milk replacement starter, 2.) molasses syrup's higher solubility (which makes it easier to apply), and 3.) molasses syrup's ability to provide enhanced explosives removal.

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ABBREVIATIONS

A1	The Anaerobic Gravel-Based Demonstration Cell at MAAP
2A-DNT	2-Amino-4,6-dinitrotoluene
4A-DNT	4-Amino-2,6-dinitrotoluene
AL	Analytical Laboratory
C	Carbon
°C	Degrees Celsius
Ca	Calcium
Cd	Cadmium
COD	Chemical Oxygen Demand
CRREL	Cold Regions Research and Engineering Laboratory
Cu	Copper
2,6-DANT	2,6-Diamino-4-nitrotoluene
2,4-DANT	2,4-Diamino-6-nitrotoluene
1,3-DNB	1,3-Dinitrobenzene
DN-4,4-AZT	Dinitro-4,4'-azoxytoluene
3,5-DNA	3,5-Dinitroaniline
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
DO	Dissolved Oxygen
EC	Electrical Conductivity
ESTCP	Environmental Security Technology Certification Program
Fe	Iron
FIA	Flow Injection Analyzer
gpm	Gallons per Minute
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High Performance Liquid Chromatography
hr	Hour
H_2SO_4	Sulfuric Acid
IC	Ion Chromatograph
ICP	Inductively Coupled Plasma
L	Liters
LIMS	Laboratory Information Management System
MAAP	Milan Army Ammunition Plant
MDL	Method Detection Limit
Mg	Magnesium
mg	Milligrams
mg/L	Milligrams per Liter
min	Minute
ml	Milliliter
Mn	Manganese
m-RDX	Mononitroso RDX
MRS	Milk Replacement Starter
mV	millivolt
µohm/cm	pico-ohms per centimeter
NH_4-N	Ammonium Nitrogen
Ni	Nickel

ABBREVIATIONS (Continued)

NO_3	Nitrate
(NO_3+NO_2)-N	Nitrate + Nitrite Nitrogen
NPOC	Non-Purgeable Organic Carbon
ORP	Oxidation-Reduction Potential
P	Phosphorus
Pb	Lead
PDA	Photodiode Array
PO_4	Orthophosphate
PO_4-P	Orthophosphate - Phosphorus
ppb	Parts Per Billion
PVC	Polyvinyl Chloride
QA	Quality Assurance
QC	Quality Control
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TKN	Total Kjeldahl Nitrogen
TN-2,2-AZT	Tetranitro-2,2'-azoxytoluene
TN-2,4-AZT	Tetranitro-2',4-azoxytoluene
TN-4,4-AZT	Tetranitro-4,4'-azoxytoluene
TNB	1,3,5-Trinitrobenzene
TNT	2,4,6-Trinitrotoluene
t-RDX	Trinitroso RDX
TVA RM	Tennessee Valley Authority Resource Management
USAEC	U.S. Army Environmental Center
WES	Waterways Experiment Station
YSI	Yellow Spring Incorporated
Zn	Zinc

SECTION 1.0
INTRODUCTION

To demonstrate the phytoremediation of explosives-contaminated groundwater, a wetlands demonstration facility has been operating at the Milan Army Ammunition Plant (MAAP) since June 17, 1996.[Ref. 1] The facility was constructed to demonstrate and validate two types of wetland systems: a gravel-based subsurface-flow system and a lagoon-based surface-flow system. The demonstration's preliminary test results indicate that the gravel-based system is the preferred wetland type.[Ref. 1] Consequently, operation of the lagoon-based system was discontinued on September 16, 1997. The gravel-based demonstration system will be operated through fiscal year 1998.

While the gravel-based system has proved to be both a technical and economic success, it was felt that further study of the gravel-based system could lead to improvements to the system's design, operation, and cost. To explore these options, three short-term studies were conducted at MAAP demonstration facility during the summer of 1997 using four 40-ft^3 anaerobic test cells. Two of the studies were designed to evaluate the use of alternative carbon sources, molasses syrup and sewage slurry, as less costly organic carbon sources. The third study was conducted to evaluate the influence of hydraulic retention time (HRT) on explosives removal. The goal of the third study was to determine if commercial-sized cells could be constructed with lower retention times. The studies were conducted using the gravel-based demonstration system's design and operating parameters as study control conditions. This report describes the results of these three studies. The report is applicable to gravel-based systems only.

The gravel-based demonstration system's design and operating parameters were developed from a series of batch-loaded and flow-through treatability studies.[Refs.2,3] The subsurface-flow gravel-based demonstration system at MAAP is a two-celled system consisting of a 17,000-ft^3 anaerobic gravel bed followed by a 5,800-ft^3 aerobic gravel bed (the aerobic cell is a TVA patent-pending process). When operating the gravel-based system, explosives-contaminated groundwater is continuously pumped at a rate of 5 gallons per minute (gpm) into a water distribution header at the front end of the anaerobic cell. The primary purpose of the anaerobic cell is to remediate explosives and explosive by-products. The anaerobic cell was designed for a 7.5-day hydraulic retention time; however, hydraulic tracer tests conducted during the

demonstration indicated the cell's actual retention time was 8.4 days. Determining if the anaerobic cell's retention time could be lowered was one of the goals of this report.

Effluent from the anaerobic cell overflows into the aerobic cell. The primary purpose of the aerobic cell is to treat the incoming water in order to:

- Remove nutrients
- Remove residual explosives and explosive by-products
- Lower the water's biochemical oxygen demand

The aerobic cell was designed for a hydraulic retention time of 2.5 days; however, during the demonstration, the aerobic cell was operated at a retention time of 1.7 days. The aerobic cell is relatively small and works well; so, improving the performance of this cell was not a subject of this report.

Both of Milan's gravel-based cells are planted with emergent plants that degrade explosives via plant enzyme nitroreductase and anaerobic microbial activity. The emergent plants used in the demonstration were: canary grass (*Phalaris arundinacea*), wool grass (*Scirpus cyperinus*), sweetflag (*Acorus calamus*) and parrotfeather (*Myriophyllum aquaticum*).

The combination of the plant enzyme nitroreductase and the subsurface anaerobic conditions results in rapid degradation of 2,4,6-Trinitrotoluene (TNT), Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and their constituent by-products via reductive pathways.[Refs. 4,5] Currently, the gravel-based system is reducing total nitrobody concentration from approximately 9,200 parts per billion (ppb) to below the demonstration goal of 50 ppb.[Ref. 1]

An important aspect of the gravel-based system's operation is the addition of an organic supplement, milk replacement starter (MRS), to the anaerobic cell. Since TVA personnel visited the MAAP site at two-week intervals, MRS amendment was added at two-week intervals during the demonstration. MRS was used during the demonstration because of its outstanding properties. MRS is an excellent carbon source:

- It provides high nutrient levels and reducing conditions (redox potential < -250 millivolts).
- It is an excellent source of the major fertilizer nutrients and trace elements which enhance plant and microbial growth.
- It is a labile carbon source which promotes the development of anaerobic microbial populations and the subsequent reduction of explosive compounds.

Total treatment costs with MRS are low; about $1.78/thousand gallons treated (including capital, operating, and maintenance cost).[Ref.1] However, it may be possible to further reduce these costs by switching carbon sources. Further improvement is possible because:

- MRS's low solubility forces operating personnel to manually prepare it as an aqueous slurry prior to adding it to the wetlands.
- Lower cost carbon sources are available.

The ideal carbon source would have physical properties that enable automated addition and be as economical to use as MRS. The first two studies in this report were designed to determine if molasses syrup or sewage slurry could be used as alternative carbon sources."

The MAAP demonstration program was funded by the Department of Defense Environmental Security Technology Certification Program (ESTCP) and was executed under a partnering agreement among the:

- U.S. Army Environmental Center (USAEC)
- Tennessee Valley Authority Resource Management (TVA RM)
- U.S. Army Corp of Engineer's Waterways Experiment Station (WES)

USAEC, as the lead agency, selected Milan Army Ammunition Plant (MAAP), located near Milan, Tennessee, as the demonstration site. TVA RM provided technical expertise in phytoremediation for the gravel-based system and in the design, construction, and operation of the demonstration facility. WES provided technical expertise in lagoon-based systems.

SECTION 2.0
MATERIALS AND METHODS

2.1 Gravel-Based Anaerobic Test Systems

The study test system consisted of four 40-ft^3 containers, or test cells (Figure 2-1), filled with clean river gravel. The clean river gravel in each container was inoculated with a small volume of microbial-rich gravel from the demonstration system's anaerobic cell (cell A1) to hasten development of microbial populations. Based on a gravel porosity of 45%, each test cell held approximately 108 gallons of water. All of the test cells were planted with a mixture of canary grass (*Phalaris arundinacea*), wool grass (*Scirpus cyperinus*), and sweetflag (*Acorus calamus*) at densities similar to that of cell A1. Because parrotfeather (*Myriophyllum aquaticum*) did not thrive in the demonstration system, it was not used during the study. The test cells were placed near the influent end (front end) of cell A1 (Figure 2-2) of the MAAP demonstration system. Placement of the test cells proximate to the influent of cell A1 allowed effluent from the test cells to flow directly into the demonstration cell, eliminating concerns about post-treatment of the test cell effluent. Placement at this location also significantly reduced the complexity of test cell design by eliminating the need for an autonomous water delivery system.

A slip stream of contaminated groundwater from the demonstration facility's inlet piping was pumped to each test cell at rates of 38 ml/min (Tests I and II) or 76 ml/min (Test III). These flow rates resulted in hydraulic retention times (HRT's) of 7.5 (standard) or 3.75 days (half standard), respectively. Two of the cells, designated cells 1 and 2, were maintained as controls and operated for the duration of the tests under "standard operating conditions," e.g., MRS supplementation averaging 20 g carbon/day and 7.5 days HRT. These "standard conditions" are similar to the conditions under which the demonstration cell A1 was operated for 15 months. Conditions in the other two test cells, cells 3 and 4, were altered to evaluate alternative organic carbon supplements (molasses syrup or sewage slurry versus MRS) or different HRT's (7.5 versus 3.75 days). Table 2-1 summarizes experimental parameters and operating conditions for the three tests conducted during this study.

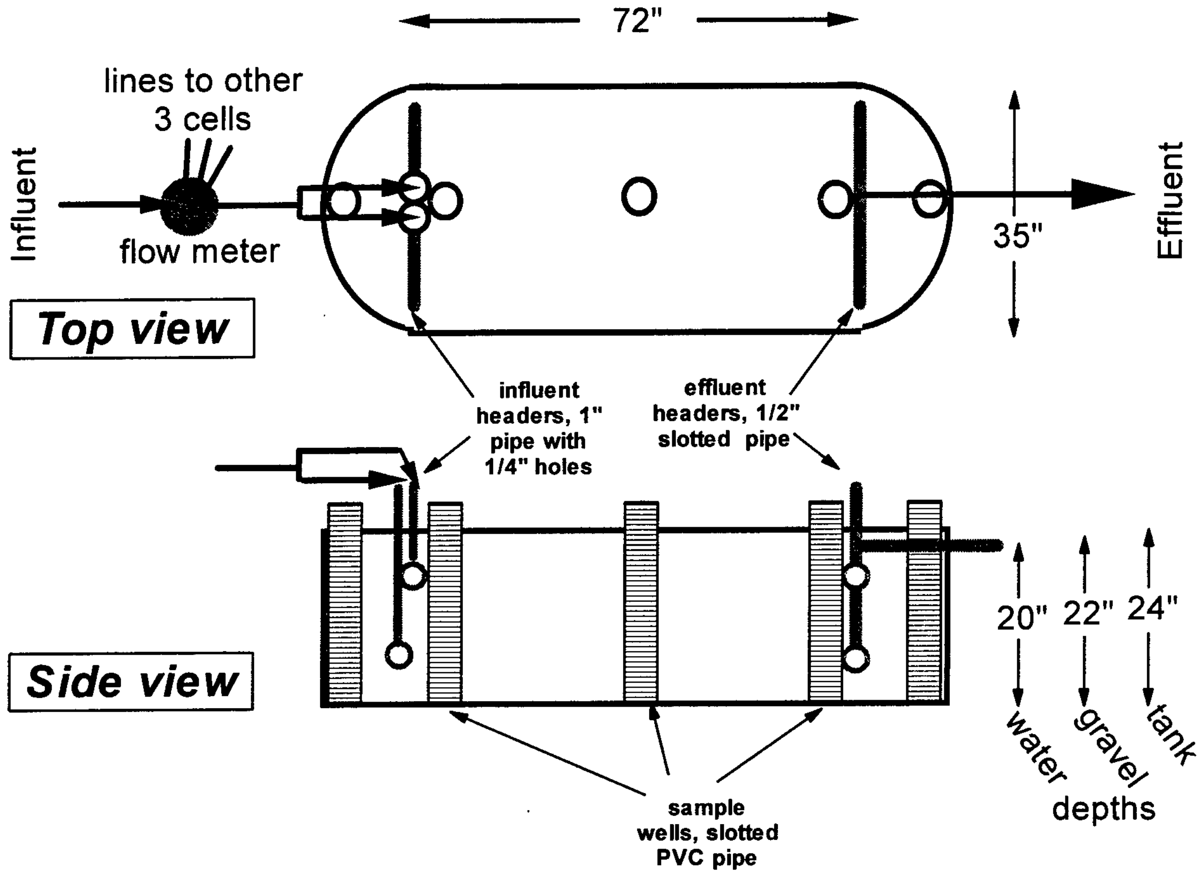

Figure 2-1

Diagram of Experimental Test Cell

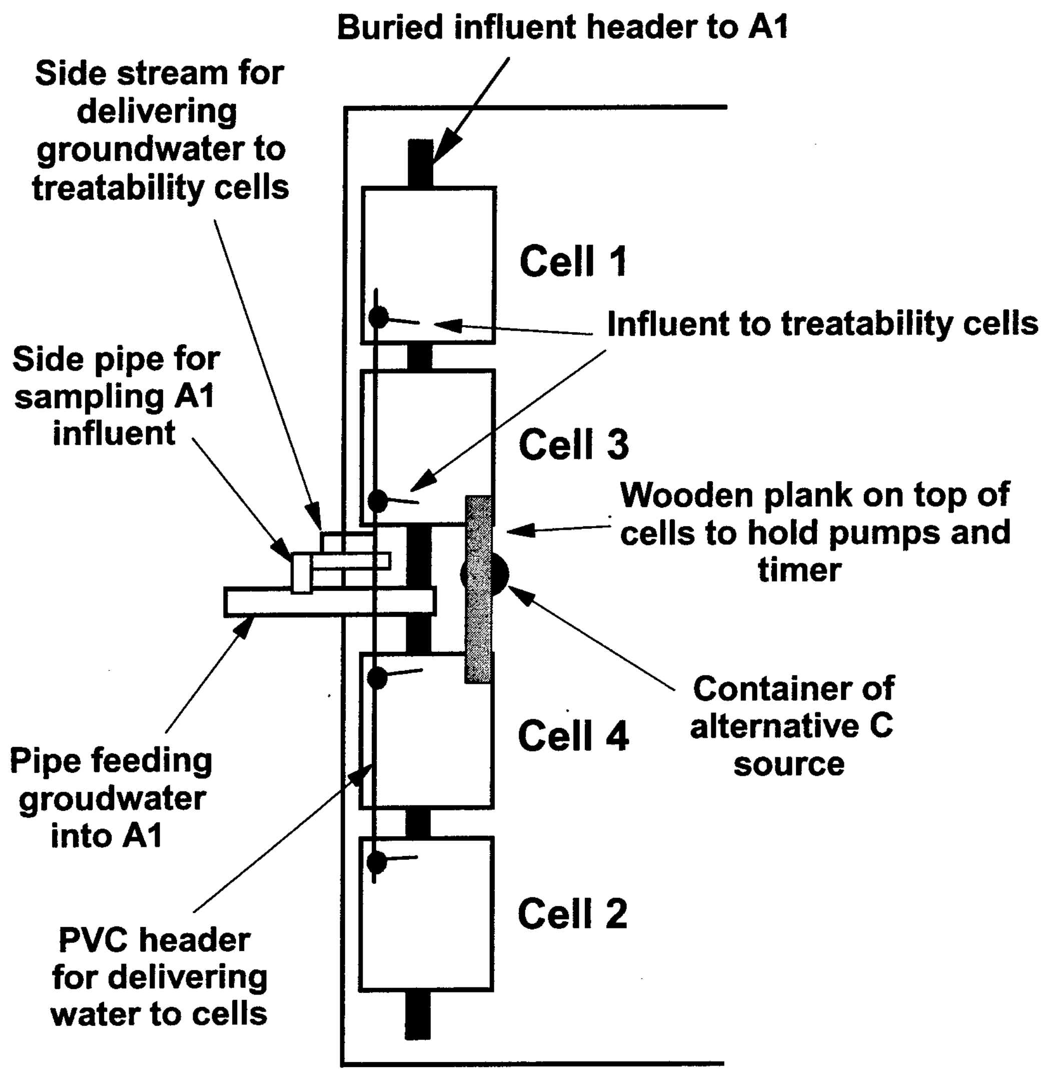

Figure 2-2

Placement of Test Cells in Demonstration Cell A1

Table 2-1

Experimental Operating Conditions and Parameters

Test	Test Cell	Water Treatment Conditions			Carbon Source		
		Retention Time (Days)	Flow Rate (ml/min)	Test Length (Weeks)	Source Type	Feed Rate (g C/day)	Dose Rate
Carbon Source - Syrup (Test I)	1	7.5	38	4	Milk Replacement Starter	20	Every 2 weeks
	2	7.5	38	4	Milk Replacement Starter	20	Every 2 weeks
	3	7.5	38	4	Syrup	20	Every 12 hours
	4	7.5	38	4	Syrup	20	Every 12 hours
Carbon Source - Sewage (Test II)	1	7.5	38	4	Milk Replacement Starter	20	Every 2 weeks
	2	7.5	38	4	Milk Replacement Starter	20	Every 2 weeks
	3	7.5	38	4	Sewage	20	Every 12 hours
	4	7.5	38	4	Sewage	20	Every 12 hours
High Flow Rate (Test III)	1	7.5	38	2	Milk Replacement Starter	20	Every 2 weeks
	2	3.75	76	2	Milk Replacement Starter	20	Every 2 weeks
	3	7.5	38	2	Syrup	20	Every 12 hours
	4	3.75	76	2	Syrup	20	Every 12 hours

2.2 Test I: Use of Molasses Syrup (Sucrose) as an Alternative Carbon Source

During Test I, use of molasses syrup (50% sucrose) was evaluated as an alternative carbon source. Carbon amendments (molasses versus MRS) were added to the test cells based on an identical carbon loading rate, averaging 20 g carbon/cell/day (Table 2-1). Cells 1 and 2 were "fertilized" with MRS, while molasses syrup was used to fertilize test cells 3 and 4.

All operating conditions were similar for the two treatments with two exceptions. First, due to differing water solubilities, the carbon sources were added at different intervals. Second, a supplemental nitrogen and phosphorus source was added to the molasses-bearing treatment systems to match the nutrient content of MRS. Due to its low solubility, MRS was batch-loaded into cells 3 and 4 at two-week intervals. In contrast, the more soluble molasses syrup was added to cells 1 and 2 in small increments throughout the day. A time-activated piston pump was used to add the molasses syrup to the cells. Five grams of monoammonium phosphate (27% P) were added to the cells containing molasses to serve as a source of ammonia nitrogen and phosphorus. MRS powder contains phosphorus and protein nitrogen which degrades to ammonium during mineralization.

The molasses syrup/MRS test was conducted over a four-week period commencing in June 1997 and ending in July 1997. Two sets of whole-column water samples were collected over the four-week period. One set of samples was collected after the second week of operation. The second sample set was collected at the end of four weeks. The samples were collected from slotted vertical sampling wells near the inlet, midpoint, and effluent positions (Figures 2-1 and 2-3). Each sample was analyzed for explosives, explosive by-products, nutrient content, and other parameters (Table 2-2). Detailed sampling procedures are described in Section 2.5.

2.3 Test II: Use of Sewage Slurry as a Carbon Source

During Test II, sewage slurry was tested as a potential carbon source. The sewage slurry consisted of groundwater inoculated with sewage sludge biosolids. The sewage sludge biosolids were obtained from a filter press located at the Moccasin Bend wastewater

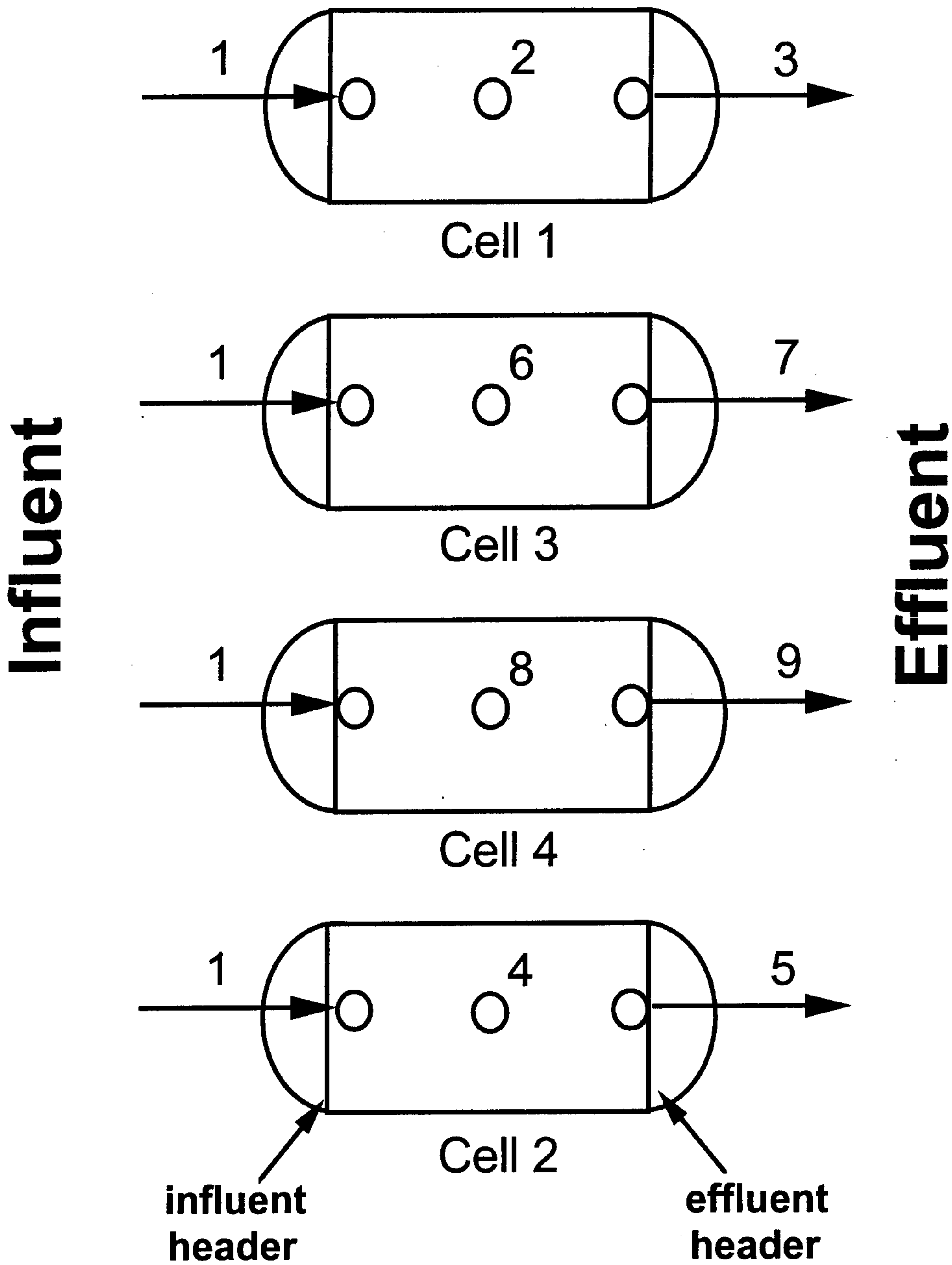

Figure 2-3

Location of Slotted PVC Sample Ports for Collecting Influent, Midpoint, and Effluent Water Samples

Table 2-2

Sampling Parameters for Determining Treatment System Efficiency

Water Quality Parameters	Frequency	Method[1]	Position Number[2]
Explosives (Total Nitrobodies)			
TNT	At end of week 2 & week 4	AP-0062	1-9
RDX	At end of week 2 & week 4	AP-0062	1-9
TNB	At end of week 2 & week 4	AP-0062	1-9
HMX	At end of week 2 & week 4	AP-0062	1-9
2,4-DNT	At end of week 2 & week 4	AP-0062	1-9
2,6-DNT	At end of week 2 & week 4	AP-0062	1-9
Explosives By-Products			
2A-DNT (TNT By-Product)	At end of week 2 & week 4	AP-0062	1-9
4A-DNT (TNT By-Product)	At end of week 2 & week 4	AP-0062	1-9
2,6-DANT (TNT By-Product)	At end of week 2 & week 4	AP-0062	1-9
2,4-DANT (TNT By-Product)	At end of week 2 & week 4	AP-0062	1-9
3,5-DNA (TNT By-Product)	At end of week 2 & week 4	AP-0062	1-9
1,3-DNB (TNB By-Product)	At end of week 2 & week 4	AP-0062	1-9
Mononitroso RDX (RDX By-Product)	At end of week 2 & week 4	AP-0062	1-9
Trinitroso RDX (RDX By-Product)	At end of week 2 & week 4	AP-0062	1-9
Azoxy Compounds	At end of week 2 & week 4	AP-0062	1-9
Test Cell Monitoring			
pH, Dissolved Oxygen (DO), Temperature, and Electrical Conductivity (EC)	At end of week 2 & week 4	Meter[3] (YSI 600 sonde)	1-9 at mid-depth
Oxidation-Reduction Potential (ORP)	At end of week 2 & week 4	Method 2580	1-9 at mid-depth
Other			
Non-Purgeable Organic Carbon (NPOC)	At end of week 2 & week 4	415 Series	1-9
Total Chemical Demand (COD)	At end of week 2 & week 4	410 Series	1-9
Plant Nutrients			
Ammonia Nitrogen (NH_4-N)	At end of week 2 & week 4	350 Series	1-9
Total Kjeldahl Nitrogen (TKN)	At end of week 2 & week 4	351 Series	1-9
Nitrate and Nitrite Nitrogen ((NO_3+NO_2)-N)	At end of week 2 & week 4	353 Series	1-9
Orthophosphate (PO_4-P)	At end of week 2 & week 4	AP-0060	1-9

1) See Appendix B of test plan for details on methods and procedures.
2) See location of sampling positions in Figure 3-2.
3) Meter methods: pH method 150.1; DO method 360.1; temperature method 170.1; EC method 120.1; and ORP (measured by method 2580).

treatment plant in Chattanooga, Tennessee. The sludge samples were originally collected for an unrelated TVA project. The sewage sludge biosolids were transported from Chattanooga to TVA's wetlands facility in Muscle Shoals, Alabama, by TVA personnel. The sludge was stored in 5-gallon containers. Upon arrival at Muscle Shoals, aliquots of the sewage sludge were obtained and the remainder was refrigerated. The aliquots were analyzed for: total nitrogen, ammonia nitrogen, total phosphorus, potassium, non-purgeable organic carbon (NPOC), metals, pH, electrical conductivity, and moisture content. When needed for the MAAP project, the sewage sludge was mixed with water to form a slurry, stored in 5-gallon containers, and transported to MAAP at room temperature and in the custody of TVA personnel.

The test with sewage slurry was initiated shortly after the test with molasses syrup (Test I) was complete. In an effort to reduce treatment carryover effects, the water in each of the test cells was drained and replaced with fresh water from well MI-051. Well MI-051 was the groundwater source for the wetlands system.

Operational procedures used during the test were the same as those indicated above for the molasses syrup test (Test I) except the sewage slurry was batch-loaded at two-week intervals rather than on a daily basis. The amount of sewage slurry pumped into test cells 3 and 4 was based on the slurry's carbon content, with the sewage carbon-loading rate being equivalent to the carbon-loading rate for treatments with the molasses syrup and MRS (Table 2-1).

The sewage slurry/MRS comparative study was conducted over a four-week period commencing in July 1997 and ending in August 1997. Two sets of whole-column water samples were collected over the four-week period. One set of samples was collected after the second week of operation. The second sample set was collected at the end of four weeks. The samples were collected from slotted vertical sampling wells near the inlet, midpoint, and effluent positions (Figures 2-1 and 2-3). Each sample was analyzed for explosives, explosive by-products, nutrient content, and other parameters (Table 2-2). Detailed sampling procedures are described in Section 2.5.

Table 2-3

Sample Parameters and Methods for Determining Wastewater Characteristics

Parameters	Frequency	Method
Explosives		
TNT	Once	AP-0062
RDX	Once	AP-0062
TNB	Once	AP-0062
HMX	Once	AP-0062
2,4-DNT	Once	AP-0062
2,6-DNT	Once	AP-0062
Explosives By-Products		
2A-DNT (TNT By-Product)	Once	AP-0062
4A-DNT (TNT By-Product)	Once	AP-0062
2,6-DANT (TNT By-Product)	Once	AP-0062
2,4-DANT (TNT By-Product)		
3,5-DNA (TNT By-Product)		
1,3-DNB (TNB By-Product)		
Mononitroso RDX (RDX By-Product)	Once	AP-0062
Trinitroso RDX (RDX By-Product)	Once	AP-0062
Azoxy Compounds	Once	AP-0062
Other		
Metals (As, Ca, Cd, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Se, Zn)	Once	200 Series
Non-Purgeable Organic Carbon (NPOC)	Once	415 Series
Chemical Oxygen Demand (COD)	Once	410 Series
Plant Nutrients		
Total Kjeldahl Nitrogen (TKN)	Once	351 Series
Total Phosphorus	Once	Lachat 10-115-10-1-C

2.4 Test III: Impact of Hydraulic Retention Time, Molasses Syrup Use, and MRS Use on Treatment Efficacy

During Test III, treatment efficacy was evaluated at two flow rates while simultaneously comparing the use of MRS and molasses syrup as carbon sources (Table 2-1). Test III was conducted over a four-week period commencing in August 1997 and ending in September 1997. Test cells 1 and 2 were operated with standard dosing of MRS (20 g carbon/day). The flow rate in cell 1 was maintained at the standard 38 ml/min while the flow rate in cell 2 was increased to 76 ml/min. Test cells 3 and 4 were operated with standard dosing of molasses syrup (20 g carbon/day). In addition to molasses syrup, 5 grams of monoammonium phosphate (27%P) was added as a source of ammonium and phosphorus (MRS contains N and P, but molasses syrup does not). The flow rate in test cell 3 was maintained at the standard 38 ml/min, while the flow rate in test cell 4 was increased to 76 ml/min. Each of the four test cells were subjected to different carbon/flow rate combinations, thus, no replication was possible during this final test. Except for the increased flow rates in cells 2 and 3, operational procedures used during Test III were the same as those indicated above for Test I.

As in the previous two tests, two sets of whole-column water samples were collected over a four-week period. One set of samples was collected after the second week of operation. The second sample set was collected at the end of four weeks. The samples were collected from slotted vertical sampling wells near the inlet, midpoint, and effluent positions (Figures 2-1 and 2-3). Each sample was analyzed for explosives, explosive by-products, nutrient content, and other parameters (Table 2-2). Detailed sampling procedures are described in Section 2.5.

2.5 Collection of Water Samples and Monitoring of Environmental Parameters

Ambient conditions were monitored during all three tests and included: water flow rate, dissolved oxygen content, pH, temperature, oxidation-reduction potential (ORP), and electrical conductivity (EC). Treatment efficacy was assessed by analysis of explosives degradation, explosives by-product content, chemical oxygen demand (COD), non-purgeable organic carbon (NPOC), and plant nutrient levels. Sewage sludge characteristics were assessed by analysis for total nitrogen, ammonia nitrogen, total phosphorus, potassium, NPOC, metals, pH, electrical conductivity, and moisture content.

The number of water sample sets collected from each test cell during each of the three tests was held constant. One set was collected after two weeks of operation and the second at the end of the respective four-week period. A summary outlining the analytical parameters and procedures is provided in Table 2-2. In addition, just prior to the sewage treatment test (Test II), a sample of the sewage was obtained and analyzed for the parameters listed in Table 2-3.

The locations of the sampling points used during the three tests are illustrated in Figure 2-3. Sample positions 1 through 9 refer to:

- A composite sample of the influents to each test cell (sample point 1)
- The sampling well located at the midpoint of each test cell (sample points 2, 4, 6, 8)
- The effluents of each test cell (sample points 3, 5, 7, 9)

The water samples collected at points 1-9 were analyzed for explosives content, explosives by-product content, nutrient content (i.e., ammonium, nitrate, and phosphate levels), pH, ORP NPOC, COD, DO, EC, and temperature. The explosive analytes included:

- 2,4,6-Trinitrotoluene (TNT)
- Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)
- Trinitrobenzene (TNB)
- Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)
- 2,4-Dinitrotoluene (2,4-DNT)
- and 2,6-Dinitrotoluene (2,6-DNT)

The total nitrobody content is defined as the sum of the total concentration of the explosives listed above.

The TNT by-product analytes included:

- 2-Amino-4,6-dinitrotoluene (2A-DNT)
- 4-Amino-2,6-dinitrotoluene (4A-DNT)

- 2,6-Diamino-4-nitrotoluene (2,6-DANT)
- 2,4-Diamino-6-nitrotoluene (2,4-DANT)
- 3,5-Dinitroaniline (3,5-DNA)
- Azoxy compounds

Analysis for azoxy compounds were included because these compounds are toxic and their presence suggests different degradation pathways. The specific azoxy compounds sought were:

- Tetranitro-2,2'-azoxytoluene (TN 2,2-AZT)
- Tetranitro-2',4-azoxytoluene (TN 2,4-AZT)
- Tetranitro-4,4'-azoxytoluene (TN 4,4-AZT)
- Dinitro-4,4'-azoxytoluene (DN 4,4-AZT)

RDX-related by-product analytes were also sought:

- Mononitroso RDX
- Trinitroso RDX

Analysis for COD and NPOC were included to determine how much residual organic carbon was released in the effluent from the test systems.

Ambient conditions in the cell were also monitored at sample positions 1 through 9 for:

- Dissolved oxygen (DO)
- pH
- Water temperature
- Electrical conductivity (EC)
- Oxidation-reduction potential (ORP)

The equipment used for collecting field data is outlined in Table 2-4. The DO, pH, water temperature, and EC measurements were collected using a hand-held sonde placed in the

Table 2-4

Equipment Used for Data Collection

Field Data	Equipment
pH	YSI 600 Sonde
Temperature	
Dissolved Oxygen (DO)	
Electrical Conductivity (EC)	
Oxidation-Reduction Potential (ORP)	Orion mV/pH Meter
Laboratory Data	
Explosives and Related By-Products	Varian HPLC
TKN, NH_4, NO_3, PO_4, and Total Phosphorus	Lachat Quick Chem 8000 or Technicon Auto Analyzer II
Non-Purgeable Organic Carbon (NPOC)	Dohrmann DC 190
Chemical Oxygen Demand (COD)	Hach DR/2000
pH	Orion Meter

sampling wells (Yellow Springs Instruments (YSI), Yellow Springs, Ohio). ORP measurements were taken with *in situ* platinum-tipped copper wire and a portable millivolt (mV) meter and calomel reference electrode.

During each sampling period, water samples were collected from sampling points 1 through 9 (Figure 2-3) located in the test cells. Water from sampling point 1 was collected as a composite from the four inlets to each cell and was collected directly from the tubing delivering groundwater to the test cells. Effluent water (sampling positions 3, 5, 7, and 9) was collected from the effluent pipe from each test cell. The water samples collected from interior wells (sampling positions 2, 4, 6, and 8) were taken as whole water column samples with a coliwasa tube.

In all sampling events, approximately 300 ml of water was collected and placed in a stainless steel beaker. Subsequently, an aliquot was transferred to a 60-ml amber glass bottle wrapped in aluminum foil. This sample underwent analysis for explosives and explosive by-products. A second aliquot of the sample was transferred to a 60-ml plastic bottle. This sample underwent COD analysis (to be conducted within 24 hours of collection). A third aliquot of the sample was transferred to a 120-ml plastic bottle. This sample underwent nutrient analyses. The third sample was preserved with 1.1 ml of 1 N H_2SO_4. All collection containers were placed in an ice chest containing ice or a commercial ice substitute and transported to TVA's laboratory in Muscle Shoals, Alabama, in the custody of a TVA employee. The samples were refrigerated upon arrival at the lab. All samples were handled in accordance with TVA's chain of custody procedures (Appendix A-14).

After collecting the water samples, the water temperature, pH, DO, EC, and ORP of the system's water were measured at all sampling positions with portable probes at mid-depth in each sampling well. Data values were recorded on a data collection sheet in the field.

Prior to conducting the test with sewage slurry, samples of the sewage sludge biosolids were analyzed for: total nitrogen, ammonia nitrogen, total phosphorus, potassium, NPOC, metals, pH, electrical conductivity, and moisture content. These samples had been collected and analyzed by TVA as part of an unrelated project. The analytical results were forwarded to TVA's technical manager.

The equipment used for collecting laboratory data is outlined in Table 2-4. Explosive and explosive by-product content were in water determined by high performance liquid chromatography. TKN, NH_4-N, NO_3-N, and PO_4-P were determined colorimetrically via an automatic analyzer. COD was determined by a colorimetric analysis. The pH of water samples taken to the laboratory was analyzed with a glass electrode and pH meter.

All analytical procedures are referenced in Appendix A1 through A-14.

SECTION 3.0
QUALITY ASSURANCE

3.1 Introduction

TVA's Analytical Laboratory (AL) at Muscle Shoals, Alabama, provided analytical chemistry support for the demonstration by performing analyses for explosives, nutrients, metals, and NPOC.

AL also provided extension and development of existing explosives analysis procedures to add more analytes to previously developed techniques.

COD analyses were performed at the TVA's Wetlands laboratory at Muscle Shoals, Alabama.

3.2 General Information

3.2.1 Project Organization and Responsibilities

The TVA Project Manager provided overall direction for the demonstration.

The TVA Wetlands Manager reported to the Project Manager and was responsible for providing technical direction and staff for development of processes and experimental design. He also provided oversight of field operations and produced the final data evaluation.

TVA's Wetlands facility staff members (Muscle Shoals) reported to the TVA Wetlands Manager and were responsible for design, operation, and assessment of the test facility.

TVA's Field Operation Team (Milan) reported to the TVA Wetlands Manager and were responsible for the operation of test facilities and documentation of experiments. The team provided for calibration and operation of test equipment. The team performed field sampling, packaged samples for shipment to the analytical laboratory, and documented sampling activities.

TVA's Laboratory Manager was responsible for providing oversight of activities in the analytical laboratory and for review of analytical laboratory data.

TVA's Quality Assurance Officer of AL reported to the Laboratory Manager and was responsible for auditing actions and documentation to ensure adherence to this plan. The TVA QA Officer was responsible for providing quarterly quality control data reports to the laboratory manager.

3.2.2 Research Records

Laboratory records from the project consist of data reports, bound research logbooks, instrument logs, worksheets, machine printouts with annotations, chromatograms, plots, review notes, and data summaries. These records have been accumulated in order by the work order number assigned by the laboratory's database and will be archived in the TVA RM records storage facility in Muscle Shoals, Alabama, for three years following the end of the project. Records are available for review at the request of USAEC.

3.2.3 Sample Custody

Field samples were handled in accordance with AL procedure SP-0001, "Sample Chain of Custody." Samples were taken in accordance with procedures described in Section 2.5. Sample custody sheets were completed at the time of sampling and delivered to the laboratory with the samples. Any problems involving broken or missing samples were handled with the sampling team and documented on the custody sheets or other receiving records.

3.3 Analytical Procedures

A written TVA procedure for explosives analysis was produced in the course of this project. The method has been peer-reviewed and is attached as Appendix A-1. It involved analysis by HPLC.

3.3.1 Nutrients, Oxygen Demand, and Metals

Other analyses for nutrients, oxygen demand, and metals were performed in accordance with standard EPA procedures, as documented in the project test plan [Ref.6] (see also procedures listed in Appendix A).

3.3.2 HPLC Analysis

The starting point for analysis of explosives and explosive degradation products for this project was EPA Method 8330, a high performance liquid chromatography (HPLC) analysis method which utilizes a methanol/water mobile phase and a UV detector. Method 8330 specifies confirmation of compounds by analyzing them on two different columns. Compounds found to be present on both columns at the correct retention time are reported as present.

Modifications to this procedure by TVA included the use of a concentration step with a Waters Porapak® RDX Sep-Pak® Vac cartridge. The dual column confirmation was replaced by analysis on a system with a photodiode array (PDA) detector, as well as on a system with a UV detector. The PDA provides an ultraviolet spectrum which can be used to confirm the identity of a compound, but it is not as sensitive as the UV detector. The same analytical column is used on both systems.

Some compounds studied in this project were additions to the analyte list in Method 8330. It was found they could not be analyzed without modification to 8330 because of co-elution problems. Scientists at the Cold Regions Research and Engineering Laboratory (CRREL) had developed an HPLC gradient method for analysis of explosives which is a modification to 8330 which uses an isocratic mobile phase.[Ref. 7] This gradient method was able to separate the target compounds for this project with one exception, so it was adopted. T-RDX and 2,6-DANT were found to co-elute, but they can be differentiated by their UV spectra. On the occasions when they were both found in a sample, the t-RDX was quantified since the detector's response is more sensitive to this compound and 2,6-DANT was reported as "present."

Water samples were either directly injected or passed through a RDX Sep-Pak column and eluted with acetonitrile which was diluted 1:1 with water, depending on the initial concentration of target compounds. All sample fractions run on the PDA were passed through a RDX Sep-Pak and eluted with acetonitrile which was diluted 1:1 with water.

3.4 Data Reduction, Validation, and Reporting

3.4.1 Data Reduction

Data from HPLC analysis of explosives and degradation products were calculated and reduced on Varian's Star workstation software which provided peak identification and peak-height calculations. Photodiode-array spectra were analyzed and compared with the same software package. Curve fitting for calibration curves was performed on an Excel spreadsheet using linear regression functions provided with that program. The resulting coefficients were applied to peak heights in a QBASIC program written at TVA RM which also reformats information to be placed into the Laboratory Information Management System (LIMS) for calculation of percent recovery of quality control samples. The LIMS software also calculates percent recovery of matrix spikes and relative percent difference between duplicate analyses.

Data from the flow injection analyzer (nitrate, ammonia, total nitrogen, etc.) were reduced and calculated using the Omnion software package on the QuikChem analyzer. These results were interfaced directly with the LIMS. This software package measures peak area and automatically applies linear regression analysis of calibration curves to determine concentrations. Percent recovery and relative percent difference for quality control samples were calculated on a spreadsheet developed at TVA RM.

Data from metals analysis were analyzed using Thermo Jarrell Ash's Enable software package which measures photomultiplier response and automatically applies linear regression analysis of calibration curves to determine concentrations. Percent recovery for quality control samples was calculated on the LIMS in the same manner HPLC data were calculated.

Data from simple instrumental methods, such as NPOC and COD, were reduced by hand or on simple spreadsheets.

3.4.2 Data Validation

Throughout the course of the project, analytical measurements were first reviewed by the chemist producing them and then by another chemist before being interfaced with the LIMS. If quality control samples fell outside limits, associated project samples were coded as "qualified" data or the samples were scheduled for reanalysis. After questions were resolved, results were passed to the TVA Laboratory Manager for final review and validation of the data packages. Additional reviews were performed by the TVA Quality Assurance Officer.

3.4.3 Data Reporting

After approval, data were reported to the TVA Wetlands Manager from the LIMS.

3.4.4 Records Retention

Records of laboratory measurements and analyses will be maintained for a period of three years after the end of the project in TVA's Muscle Shoals Records Center. This is a federal agency record center with access control, retrieval, and fire protection, as described in 36 CFR 1228 Subpart K.

All analytical data were accumulated as packages from each sampling event. Each package included as a minimum: sample descriptions or identification information, a copy of the chain of custody record, sample analytical results, quality control sample results with percent recovery of the added compounds, worksheets, chromatograms, raw data, and a copy of the final report. Data from failed attempts at measurement were stored, along with other records for samples.

Support records were also accumulated which included determination of method detection limits, records of purchase of standard materials, and records of use of standard materials.

3.4.5 Qualification Codes

The following codes may be found in data packages.

- NA - Compound not analyzed.
- <MDL - Compound not detected [analysis value falls below the method detection limit (MDL)].
- TR - Compound was present at trace level. Indicated but less than MDL.
- Q - "Qualified" - For a sample in which an analyte was found, the measurement for an associated quality control sample for that same analyte fell outside control limits.

3.5 Internal Quality Control

3.5.1 Initial Quality Control

AL routinely ran blank samples to demonstrate that glassware and reagents were free of interferences.

Initially, and as methods were developed, quality control check sample sets of known concentration were run to ensure method precision and accuracy were known.

For automated analytical equipment, such as flow injection analyzers and high-performance liquid chromatography, retention time windows or timing windows were established in order for analytes to be properly identified by analytical software.

Each analyst demonstrated the ability to generate acceptable results with the methods before working alone on project samples.

3.5.2 Cross-Check and Blind Quality Control Samples

The laboratory routinely participated in nationally promulgated cross-checks, internally produced blind samples, and purchased quality control samples to demonstrate the laboratory's ability as compared to national performance of commonly performed methods. Results taken from internal quality control data reports are discussed below.

Sample 96-08-024 was submitted for NPOC and COD analysis.

Known NPOC	Found NPOC	% Recovery	Known COD	Found COD	% Recovery
71.9 mg C/l	61.5	86	192 mg O_2/L	195	102
				228	119
				197	103
				197	103

Sample 96-09-027 was spiked at 20.0 mg carbon/liter. NPOC analysis (NPOC) yielded 19.48 mg/liter (97% recovery).

Sample 96-09-034-01A was spiked at 290 mg carbon/liter. NPOC gave 368 mg/liter. Sample - 02A from this same work order was spiked at 345 mg/liter. NPOC gave 384 mg/liter. Yields were 127% and 111%. Problems with the device were found and corrected before project data were reported.

Work Order 96-12-059 was scheduled for metals analysis. It gave the following results:

Metal	Known mg/L	Found mg/L	% Recovery
Calcium	40.0	41.4	104
Iron	2.00	2.08	104
Lead	2.00	2.06	103
Magnesium	0.80	0.87	109

Quality control samples were purchased from Environmental Resource Associates (ERA). One setpoint Laboratory Standard for COD was purchased and delivered to the Wetlands laboratory for analysis in conjunction with this project.

Analyte	Found (mg/L)	True Value	Lower Limit (95%)	Higher Limit (95%)
COD	384	379	350	408
	380			
	381			

An ERA "WasteWatR" sample set was submitted to TVA's laboratory as Work Order 97-02-002. Results are evaluated below.

Analyte	Found mg/L or ug/L (metals)	Known	Low Limit	High Limit
COD	167	175	149	201
TKN	11	10.5	8.6	13.1
NPOC	159.4	69.9	59.34	80.34
Total P	4.38	4.23	3.64	5.5
As	78.4	76.5	57.4	90.3
Cd	84	78.6	113	
Cu	114	118	96.8	139
Fe	430	471	386	556
Pb	< LOQ	132	108	156
Mn	187	188	154	222
Hg	10.3	10.6	7.95	13.3
Ni	432	406	333	479
Se	79	97.1	72.8	115
Zn	208	221	181	261
Ammonia N by FIA	6.98	6.83	4.69	7.92
Nitrate by FIA	12.51	12.8	11.4	14.2
Phosphate by FIA	10.74	11.4	9.69	13.1

Work Order 97-01-008 was submitted for nitrate analysis by flow injection analyzer (FIA). This was a potassium nitrate solution. Ten replicate analyses gave a mean of 38.5 and a standard deviation of 0.5 ppm. Known value was 39.6 ppm (97% recovery).

A demand standard was purchased from Analytical Products Group and run as an unknown. Values were found as listed below.

Known COD (mg/L)	Limits	Found
27.3	17.3 - 35.0	24
		27

Sample 97-03-032 was a set of phosphate samples run by flow injection analyzer. Values found are tabulated below.

Known (mg P/L)	Found (FIA)
3.72	3.6
7.60	7.4
14.5	14.2

3.5.3 **Batch Quality Control**

For automated analyses, a variety of quality control samples were analyzed routinely with each batch. These included reagent blanks, midpoint calibration standards, laboratory control samples, matrix spikes, and duplicates. Percent recovery was calculated for midpoint calibration standards, laboratory control samples, and matrix spikes. Relative percent difference was calculated for duplicate samples. In all, thousands of quality control analyses were performed for this project. Typical analytical quality control for a HPLC run was as follows:

Sample Type	Frequency
Laboratory Control Sample (made from a separate stock than the calibration standards)	Every 20 field samples[1]
Method Blank	Every 20 field samples
Matrix Spike	Every 20 field samples
Matrix Spike Duplicate	Every 20 field samples
Initial Calibration Check[2]	At beginning of run
Continuing Calibration Check[2]	After every 10 injections[3]
Final Calibration Check[2]	At end of run

1) Analytical batch quality control samples were run for every 20 samples (or subset thereof) of the same matrix prepared with the same reagents on the same day.

2) Calibration check samples were injected as two solutions because of peak overlap.

3) Calibration check samples were run after every 10 injections counting field samples, method blanks, matrix spikes, matrix spike duplicates, and laboratory control samples.

Quality control for non-automated methods (COD and NPOC) was more limited. Runs included duplicates, blanks, and knowns.

3.5.4 Calibration

Calibration of flow injection analyzers, carbon analyzer, chemical demand analyzer, and inductively coupled plasma devices were made with each analytical run using software provided by the manufacturer of the device.

Calibration of the HPLC device was done initially when the column was changed and when quality control sample response indicated that recalibration was required. Calibration was done at five concentrations. Data were fit to three models: slope only ($y = mx$), linear ($y = a + bx$), and quadratic ($y = a + bx + cx^2$). The choice of the model was made based on back-calculation of the calibration standards.

3.6 Method Detection Limits

TVA's AL determined method detection limits as defined in 40 CFR Part 136, Appendix B, Revision 1.11. Detection limits were documented in internal memoranda with associated data packages. Limits were reported with analytical results. Detection limits for HPLC were found to be a function of column age and detector stability.

Typical method detection limits for explosives and explosive by-product analytes are listed in Table 3-1. For explosives and explosive by-products, effluent water and other low-concentration samples were concentrated before analysis to increase the detection limits.

Typical method detection limits for other analytes in water and their units are tabulated in Table 3-2.

3.7 Performance and System Audits

TVA's QA Officer performed internal audits: two surveillances of sampling practices at the Wetlands facility and an audit of data review practices. Results were reported in writing to the Laboratory Manager as internal audits. In addition, an initial review of the project test plan was made with the laboratory staff which was also written as an internal audit.[Ref. 6] Suitable corrective actions were instituted in response to concerns and findings of these audit reports. The corrective action tracking system utilized by the laboratory was employed to track these items to closure, as appropriate. Corrective actions included issuance or revision of procedures, change of sampling practices, revision to the project test plan, development of data review checklists, streamlining of data-handling practices, and calibration of balances.

The TVA QA Officer also inspected control charts, logs, records, printouts, results of quality control checks, and other quality-related documents from the project.

USAEC staff also reviewed procedures, interim data, and project reports. Findings and concerns from these reviews also resulted in corrective actions by the laboratory staff. As appropriate, some of these were tracked to completion on the corrective action tracking system.

Table 3-1

Method Detection Limits for Explosives and Explosive By-Products

Analyte		Influent (mg/L)	Effluent (mg/L)
Abbreviation	**Name**		
TNT	2,4,6-Trinitrotoluene (TNT)	0.005	0.0004
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine	0.006	0.0005
TNB	1,3,5-Trinitrobenzene (TNB)	0.005	0.0004
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	0.005	0.0004
2,4-DNT	2,4-Dinitrotoluene	0.005	0.0004
2,6-DNT	2,6-Dinitrotoluene	0.006	0.0005
2,6-DANT	2,6-Diamino-4-nitrotoluene	0.005	0.0004
2,4-DANT	2,4-Diamino-6-nitrotoluene	0.005	0.0004
2-DNT	2-Amino-4,6-dinitrotoluene	0.005	0.0004
4-DNT	4-Amino-2,6-dinitrotoluene	0.005	0.0004
TN 2,2'-AZT	Tetranitro-2,2'-azoxytoluene	0.005	0.0004
TN 2',4-AZT	Tetranitro-2',4-azoxytoluene	0.011	0.0008
TN 4,4'-AZT	Tetranitro-4,4'-azoxytoluene	0.008	0.0006
DN 4,4'-AZT	Dinitro-4,4'-azoxytoluene	0.014	0.001
1,3-DNB	1,3-Dinitrobenzene	0.005	0.0004
3,5-DNA	3,5-Dinitroaniline	0.005	0.0004
m-RDX	Mononitroso-RDX	0.005	0.0004
t-RDX	Trinitroso-RDX	0.005	0.0004

Table 3-2
Method Detection Limits for Nutrients and Metals

Test Description	Limit	Units
Nutrients		
Ammonia	0.02	mg NH_3-N/L
Nitrate Plus Nitrite as Nitrogen	0.08	mg NO_3-N/L
Phosphate as Phosphorus	0.01	mg PO_4-P/L
Total Kjeldahl Nitrogen	0.05	mg N/L
Non-Purgeable Organic Carbon	0.9	mg/L
Metals		
Cadmium	0.03	mg/L
Calcium	0.03	mg/L
Copper	0.02	mg/L
Iron	0.02	mg/L
Lead	0.3	mg/L
Magnesium	0.2	mg/L
Manganese	0.008	mg/L
Nickel	0.07	mg/L
Zinc	0.009	mg/L

SECTION 4.0

RESULTS AND DISCUSSION

4.1 Test I: Explosives Removal as a Function of Supplemental Carbon Source (Molasses Syrup versus MRS)

Table 4-1 summarizes the results of the molasses syrup versus MRS test (Test I). Explosives removal was extremely rapid and nearly complete in both test systems, irrespective of carbon source. Total nitrobodies (i.e., TNT, RDX, HMX, TNB, 2,4-DNT, and 2,6-DNT) in the well water influent summed to 9,006 and 8,976 ppb on sample dates July 1, 1997, and July 20, 1997, respectively. The sum of effluent concentrations of total nitrobodies on July 1, 1997, for MRS and molasses syrup treatments were 20 ppb and less than detection limits, respectively. On July 20, 1997, there were no detectable concentrations of nitrobodies in either of the test systems. Furthermore, total nitrobody concentrations in samples collected at the midpoint of the treatment systems were also very low or below detection limits (Table 4-1).

The only explosive degradation by-products to be detected on July 1, 1997, were the RDX by-products m-RDX and t-RDX; and the TNT by-products 2,4-DANT and 4 A-DNT. 20 ppb of m-RDX was observed at the mid-point of the test cells containing molasses syrup. T-RDX was observed at 132 ppb and 338 ppb in the midpoint and effluent samples in the test cells containing MRS, and at 206 and 217 ppb in the midpoint and effluent samples in test cells containing molasses syrup treatment. Low concentrations of 2,4-DANT and 4A-DNT were also observed in midpoint samples in the test cells containing molasses syrup, 88 and 20 ppb, respectively. However, the 2,4-DANT and 4A-DNT concentrations in the effluent were always below detection limits.

On the July 20, 1997, sampling date, t-RDX was observed at 17 and 5 ppb in the midpoint samples of the test cells containing MRS and molasses syrup treatments, respectively. All of the effluent explosives and explosive by-product concentrations were below the detection limits.

Table 4-1

Removal of Explosives and Explosives By-Products in a Subsurface Flow Constructed Wetland as a Function of Carbon Source (Molasses Syrup versus Milk Replacement Starter)[1]

DATE	JULY 1, 1997					JULY 20, 1997				
TREATMENT		Milk Replacement Starter		Molasses Syrup			Milk Replacement Starter		Molasses Syrup	
Parameter	Influent (ppb	Midpoint (ppb)	Effluent (ppb)	Midpoint (ppb)	Effluent (ppb)	Influent (ppb)	Midpoint (ppb)	Effluent (ppb)	Midpoint (ppb)	Effluent (ppb)
Total Nitrobodies										
TNT	4,320	MDL[2]	MDL	MDL	MDL	4,360	MDL	MDL	MDL	MDL
RDX	4,191	MDL	20.0	24.4	MDL	4,120	MDL	MDL	MDL	MDL
TNB	326	MDL	MDL	MDL	MDL	331	MDL	MDL	MDL	MDL
HMX	87.9	MDL	MDL	10	MDL	90	MDL	MDL	MDL	MDL
2,4-DNT	81.23	MDL	MDL	MDL	MDL	76	MDL	MDL	MDL	MDL
2,6-DNT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
Total	9,006	MDL	20	34.4	MDL	8,977	MDL	MDL	MDL	MDL
Explosive By-Products										
2,6-DANT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,4-DANT	MDL	MDL	14[3]	88	MDL	MDL	MDL	MDL	MDL	MDL
4A-DNT	15.5	MDL	MDL	20	MDL	MDL	MDL	MDL	MDL	MDL
2A-DNT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 2,2'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 2',4-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
DN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
1,3-DNB	12.7	MDL	MDL	MDL	MDL	15	MDL	MDL	MDL	MDL
3,5-DNA	7.52	MDL	MDL	MDL	MDL	12.4	MDL	MDL	MDL	MDL
m-RDX	11.9	MDL	26[3]	20	MDL	22	MDL	MDL	MDL	MDL
t-RDX	MDL	132	338	206	217	MDL	17	MDL	5[3]	MDL

1) Each value represents the average of two replicates.
2) MDL indicates concentrations were less than the method detection limits (Table 3-1).
3) Based on a single value. The other value was below the method detection limit.

4.2 Test II: Explosives Removal as a Function of Supplemental Carbon Source (Sewage Slurry versus MRS)

Concentrations of total nitrobodies in the contaminated groundwater influent on August 5, 1997, and August 19, 1997, were 9,258 and 9,072 ppb to the test cells containing sewage slurry and MRS, respectively. As in Test I, explosives and explosive by-products were rapidly reduced to below detection limits (Table 4-2). Sampling of water from midpoint and effluent ports on both sample dates revealed that nitrobody concentrations had been reduced to less than detection limits, irrespective of treatment (Table 4-2). Low t-RDX concentrations were detected at the midpoint (98 ppb) and effluent samples (31 ppb) of the test cells containing MRS on August 19, 1997. A small amount of t-RDX (0.7 ppb) was also detected at the midpoint of the cells being treated with sewage slurry on August 19, 1997. However, the t-RDX concentrations found during this study (Test II) were generally much lower than the concentrations found during the Test I study (Table 4-1 versus Table 4-2), possibly due to increased plant biomass and/or maturation of the treatment wetlands and their respective anaerobic microbial communities.

4.3 Test III: Explosives Removal as a Function of Hydraulic Retention Time and Carbon Supplementation

This non-replicated two-factor study was conducted over a four-week period in August and September 1997. The intent of the study was to evaluate the relative impact of four treatment combinations involving HRT (7.5 versus 3.75) and carbon source supplementation (molasses syrup versus MRS) on reduction of primary explosives and explosive by-products. The treatment combination consisting of 7.5 days HRT and MRS organic supplementation (20 g carbon/unit/day) was designated as a comparative baseline for the other three factorial treatments since 7.5 days HRT and MRS supplementation have been the standard operating conditions for the Milan demonstration anaerobic wetland.

As in the previous two tests, influent concentrations of total nitrobodies were relatively high, summing to 8,954 and 9,649 ppb on sample dates September 2 and 16, 1997, respectively.

Table 4-2

Removal of Explosives and Explosive By-Products in a Subsurface Flow Constructed Wetland as a Function of Carbon Source (Sewage Slurry versus Milk Replacement Starter)[1]

SAMPLE DATE		AUGUST 5, 1997				AUGUST 19, 1997				
TREATMENT		Milk Replacement Starter		Sewage Slurry			Milk Replacement Starter		Sewage Slurry	
Parameter	Influent (ppb)	Midpoint (ppb)	Effluent (ppb)	Midpoint (ppb)	Effluent (ppb)	Influent (ppb)	Midpoint (ppb)	Effluent (ppb)	Midpoint (ppb)	Effluent (ppb)
Total Nitrobodies										
TNT	4,510	MDL[2]	MDL	MDL	MDL	4,440	MDL	MDL	MDL	MDL
RDX	4,260	MDL	MDL	MDL	MDL	4,140	MDL	MDL	MDL	MDL
TNB	336	MDL	MDL	MDL	MDL	329	MDL	MDL	MDL	MDL
HMX	90	MDL	MDL	MDL	MDL	93	MDL	MDL	MDL	MDL
2,4-DNT	62.4	MDL	MDL	MDL	MDL	70	MDL	MDL	MDL	MDL
2,6-DNT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
Total	9,258	MDL	MDL	MDL	MDL	9,072	MDL	MDL	MDL	MDL
Explosive By-Products										
2,6-DANT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,4-DANT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
4A-DNT	26.2	MDL	MDL	MDL	MDL	37.8	MDL	MDL	MDL	MDL
2A-DNT	17.7	MDL	MDL	MDL	MDL	23.4	MDL	MDL	MDL	MDL
TN 2,2'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 2',4-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
DN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
1,3-DNB	15.6	MDL	MDL	MDL	MDL	15.9	MDL	MDL	MDL	MDL
3,5-DNA	12.9	MDL	MDL	MDL	MDL	11.4	MDL	MDL	MDL	MDL
m-RDX	26.5	MDL	MDL	MDL	MDL	16.5	MDL	MDL	MDL	MDL
t-RDX	MDL	MDL	MDL	MDL	MDL	MDL	98	31	0.7	MDL

1) Each value represents the average of two replicates.
2) MDL indicates concentrations were less than the method detection limits (Table 3-1).
3) Based on one observation, second observation was below detection limits.

Tables 4-3 and 4-4 summarize concentrations of explosives and explosive by-products at influent, midpoint, and effluent positions on the respective sample dates.

Test III's results revealed that treatment efficacies were impacted by both hydraulic retention time and carbon source. Efficacy of treatment combinations (most effective to least effective) were ranked accordingly (Syrup 7.5 > Syrup 3.75 > MRS 7.5 > MRS 3.75). Figure 4-1 illustrates residual concentration of explosives and explosive by-products for both sample dates as a function of treatment combination and sample location. Short HRT's in the MRS treatments led to significantly higher residual concentrations of RDX, m-RDX, t-RDX, and HMX. Treatments receiving molasses syrup supplementation, irrespective of HRT, had significantly lower residual concentrations of explosives and explosive by-products as compared to MRS-amended treatments.

4.4 Nutrient Dynamics and Water Quality

Nutrient dynamics and water quality data for these short-term studies were comparable to results reported for earlier treatability studies[Ref. 2,3], and for the Phase II Milan demonstration.[Ref. 1] Five baseline water quality parameters were measured on dates when water samples were collected for nutrient and explosives analysis. Tables 4-5, 4-6, and 4-7 summarize mean values and standard deviations (in parenthesis) for DO, ORP, pH, EC, and temperature as a function of date, treatment, and sampling location.

Under conditions of high organic loading, such as practiced in this series of tests, plant root respiration and microbial respiration rapidly reduced dissolved oxygen concentrations to anoxic/anaerobic conditions and ultimately reduced ORP to values ranging from -292 to -505 millivolts (mV) (Tables 4-5 to 4-7). Both DO levels and ORP values were consistently lower in Tests I and II during the second sampling dates, as compared to the first sampling dates.

Table 4-3

Removal of Explosives and Explosive By-Products in a Subsurface Flow Constructed Wetland as a Function of Organic Carbon Source (Molasses Syrup versus Milk Replacement Starter) and Hydraulic Retention Time (7.5 versus 3.75 days)[1]

SAMPLE DATE	SEPTEMBER 2, 1997								
TREATMENT		MRS 7.5 days		MRS 3.75 days		SYRUP 7.5 days		SYRUP 3.75 days	
Parameter	Influent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)
Total Nitrobodies									
TNT	4,380	MDL[2]	MDL	MDL	MDL	MDL	MDL	MDL	MDL
RDX	4,080	MDL	12.7	613	24.4	MDL	MDL	MDL	MDL
TNB	320	MDL	MDL	1.93	MDL	MDL	MDL	MDL	MDL
HMX	86.9	MDL	MDL	30	1	MDL	MDL	MDL	MDL
2,4-DNT	86.6	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,6-DNT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
Total	8,954	MDL	12.7	645	25.4	MDL	MDL	MDL	MDL
Explosive By-Products									
2,6-DANT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,4-DANT	MDL	MDL	1.88	218	6.51	MDL	MDL	MDL	MDL
4A-DNT	22	MDL	MDL	10.2	MDL	MDL	MDL	MDL	MDL
2A-DNT	9.2	MDL	MDL	3.33	MDL	MDL	MDL	MDL	MDL
TN 2,2'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 2',4-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
DN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
1,3-DNB	16	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
3,5-DNA	11.7	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
m-RDX	MDL	5.22	9.32	128	9.26	MDL	MDL	MDL	MDL
t-RDX	MDL	82.3	85.6	451	324	5.21	1.2	21.9	3.46

1) Each value is based on an individual observation, i.e., no replicates.
2) MDL indicates concentrations were less than the method detection limits (Table 3-1).

Table 4-4

Removal of Explosives and Explosive By-Products in a Subsurface Flow Constructed Wetland as a Function of Organic Carbon Source (Molasses Syrup versus Milk Replacement Starter) and Hydraulic Retention Time (7.5 versus 3.75 days)

SAMPLE DATE	SEPTEMBER 16, 1997								
TREATMENT		MRS 7.5 days		MRS 3.75 days		SYRUP 7.5 days		SYRUP 3.75 days	
Parameter	Influent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)	midpoint (ppb)	effluent (ppb)
Total Nitrobodies									
TNT	4,730	MDL[2]	MDL	MDL	MDL	MDL	MDL	MDL	MDL
RDX	4,460	40.4	47.7	924	57.6	MDL	MDL	8.89	MDL
TNB	351	0.536	MDL	2.77	MDL	MDL	MDL	MDL	MDL
HMX	98.4	1.85	2.29	47.5	4.08	MDL	MDL	MDL	MDL
2,4-DNT	9.47	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,6-DNT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
Total	9,649	42.8	50.0	974	61.7	MDL	MDL	8.89	MDL
Explosive By-Products									
2,6-DANT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
2,4-DANT	MDL	12.6	12.3	299	15	MDL	MDL	8.57	MDL
4A-DNT	25.2	MDL	MDL	19.5	MDL	MDL	MDL	MDL	MDL
2A-DNT	15.6	MDL	MDL	8.33	MDL	MDL	MDL	MDL	MDL
TN 2,2'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 2',4 -AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
TN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
DN 4,4'-AZT	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
1,3-DNB	16.8	MDL	MDL	MDL	MDL	MDL	MDL	MDL	MDL
3,5-DNA	13.1	0.48	MDL	MDL	MDL	MDL	MDL	MDL	MDL
m-RDX	MDL	16.6	18.9	170	27.3	MDL	MDL	MDL	MDL
t-RDX	MDL	177	173	418	474	MDL	MDL	52.9	5.46

1) Each value is based on an individual observation, i.e., no replicates.
2) MDL indicates concentrations were less than the method detection limits (Table 3-1).

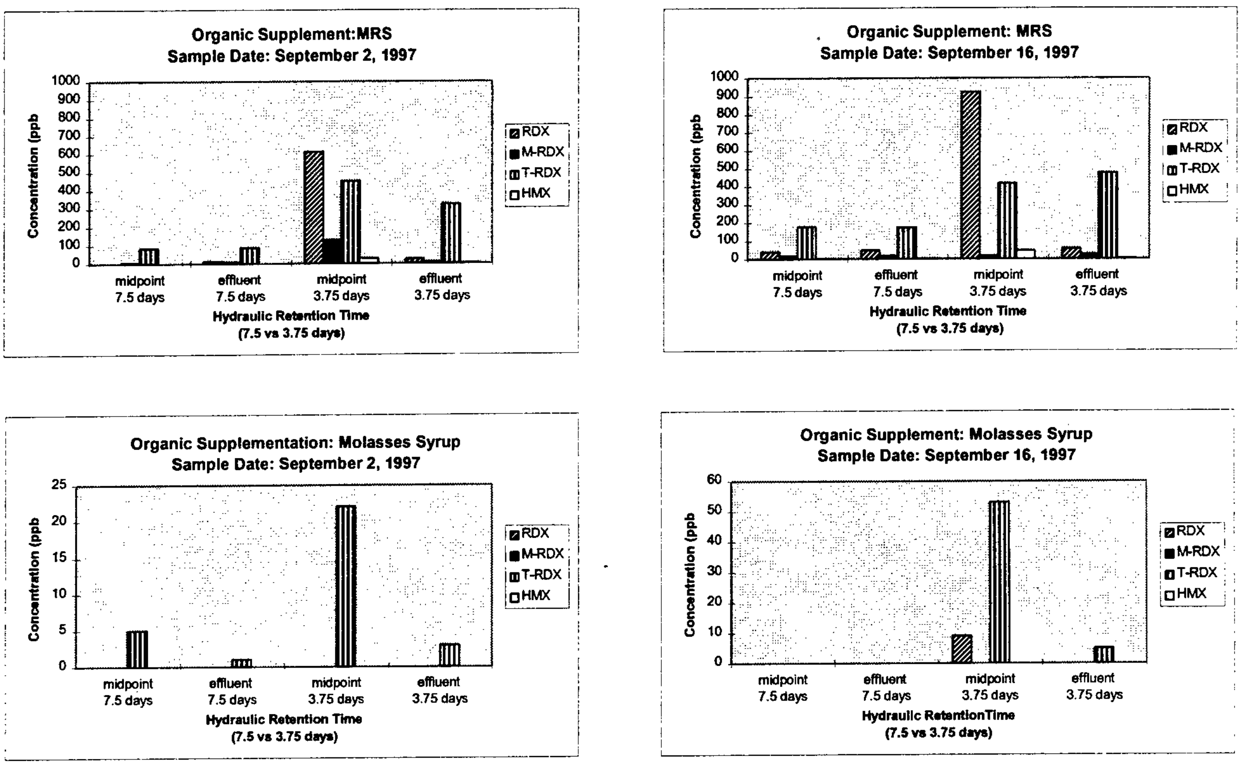

Figure 4-1

Residual Concentrations of Explosives and Explosive By-Products as a Function of Sample Date, Treatment Combination, and Sample Location[1]

1) Values are based on individual observations (n=1).

Table 4-5

Water Quality as a Function of Treatment, Sample Date, and Sample Location for Test I - Data for July 1 and July 20, 1997[1,2]

Date	Treatment	MRS	MRS	MRS	Syrup	Syrup	Syrup
	Parameter	**Influent**	**Midpoint**	**Effluent**	**Influent**	**Midpoint**	**Effluent**
7/1/97	DO (mg/L)	1.0 (0.32)	1.1 (0.54)	1.8 (1.77)	1.0 (0.39)	0.7 (0.01)	1.0 (0.28)
7/1/97	ORP (mV)	-292 (182.1	-428 (7.9)	-432 (10.1)	-378 (37.6)	-415 (20.9)	-417 (8.8)
7/1/97	pH	6.8 (0.08)	6.7 (0.04)	6.6 (0.34)	6.5 (0.06)	6.5 (0.07)	6.6 (0.02)
7/1/97	EC (µohm/cm)	644 (289.9)	865 (238.3)	850.0 (161.2)	986 (12.&0	1195 (63.6)	1045 (72.8)
7/1/97	Temp (°C)	23.1 (0.62)	23.2 (1.90)	23.9 (2.09)	21.8 (0.49)	22.8 (2.54)	23.1 (1.44)
Date	**Treatment**	**MRS**	**MRS**	**MRS**	**Syrup**	**Syrup**	**Syrup**
	Parameter	**Influent**	**Midpoint**	**Effluent**	**Influent**	**Midpoint**	**Effluent**
7/20/97	DO (mg/L)	0.7 (0.16)	0.6 (0.01)	0.5 (0.02)	0.6 (0.01)	0.6 (0.06)	0.5 (0.02)
7/20/97	ORP (mV)	-505 (0.71)	-493 (10.6)	-482 (23.3)	-488 (5.6)	-492 (10.6)	-491 (1.4)
7/20/97	pH	7.0 (0.01)	6.9 (0.00)	6.9 (0.03)	6.7 (0.0)	6.6 (0.04)	6.8 (0.12)
7/20/97	EC (µohm/cm)	690 (155.6)	726 (154.9)	745 (127.3)	990 (134.4)	998 (25.5)	998 (47.4)
7/20/97	Temp (°C)	25.9 (1.34)	25.8 (0.64)	26.5 (1.41)	23.7 (0.49)	23.6 (0.14)	24.7 (0.64)

1) Each value is based on the average of two observations (n=2).
2) Numbers in parenthesis represent one standard deviation.

Table 4-6

Water Quality as a Function of Treatment, Sample Date, and Sample Location for Test II - Data for August 6 and August 19, 1997[1,2]

Date	Treatment	MRS	MRS	MRS	Sewage Slurry	Sewage Slurry	Sewage Slurry
	Parameter	Influent	Midpoint	Effluent	Influent	Midpoint	Effluent
8/6/97	DO (mg/L)	0.6 (0.33)	0.7 (0.30)	0.5 (0.16)	0.5 (0.01)	1.0 (0.20)	0.8 (0.44)
8/6/97	ORP (mV)	-191 (238.3)	-377 (137.9)	-294 (17.7)	-174 (11.3)	-222 (66.5)	-175 (19.8)
8/6/97	pH	7.0 (0.08)	6.9 (0.02)	6.9 (0.01)	7.2 (0.37)	7.1 (0.13)	7.0 (0.01)
8/6/97	EC (µohm/cm)	554 (53.0)	664 (120.2)	669 (119.5)	571 (55.9)	677 (9.2)	724 (41.0)
8/6/97	Temp (°C)	22.3 (0.43)	22.8 (0.41)	22.8 (0.69)	21.6 (0.02)	22.5 (0.41)	22.3 (0.60)
Date	Treatment	MRS	MRS	MRS	Sewage Slurry	Sewage Slurry	Sewage Slurry
	Parameter	Influent	Midpoint	Effluent	Influent	Midpoint	Effluent
8/19/97	DO (mg/L)	0.4 (0.00)	0.4 (0.00)	0.4 (0.00)	0.4 (0.07)	0.4 (0.00)	0.5 (0.07)
8/19/97	ORP (mV)	-291 (253)	-469 (1.4)	-428 (37.5)	-343 (165.5)	-465 (0.00)	-468 (19.1)
8/19/97	pH	6.9 (0.07)	6.7 (0.00)	6.7 (0.00)	6.8 (0.14)	6.8 (0.07)	6.7 (0.00)
8/19/97	EC (µohm/cm)	514 (78.5)	671 (241.8)	695 (191.6)	623 (2.12)	697 (72.8)	928 (65.8)
8/19/97	Temp (°C)	25.5 (0.42)	26.3 (1.13)	25.2 (0.00)	24.7 (0.42)	14.8 (14.28)	26 (0.35)

1) Each value is based on the average of two observations (n=2).
2) Numbers in parenthesis represent one standard deviation.

Table 4-7

Water Quality as a Function of Treatment, Sample Date, and Sample Location for Test III - Data for September 2 and September 16, 1997[1,2]

Date	Treatment	MRS 7.5			MRS 3.75		
	Parameter	Influent	Midpoint	Effluent	Influent	Midpoint	Effluent
9/2/97	DO (mg/L)	0.6	0.6	1.2	1.2	0.5	0.8
9/2/97	ORP (mV)	-38	-388	-351	71	30	-208
9/2/97	pH	6.6	6.5	6.2	6.6	6.6	6.5
9/2/97	EC (µohm/cm)	604.0	787.5	796.5	531	558	598
9/2/97	Temp (°C)	24.5	24.7	24.5	23.0	23.6	24.5
Date	Treatment	Syrup 7.5			Syrup 3.75		
	Parameter	Influent	Midpoint	Effluent	Influent	Midpoint	Effluent
9/16/97	DO (mg/L)	0.4	0.9	0.6	2.8	0.6	1.0
9/16/97	ORP (mV)	-307	-252	-267	-213	-414	-337
9/16/97	pH	6.3	6.6	6.5	6.4	6.4	6.5
9/16/97	EC (µohm/cm)	1204	1165	1115	480	637	713
9/16/97	Temp (°C)	24.0	24.6	24.4	21.9	22.6	24.0

1) Each value is based on the average of two observations (n=2).
2) Numbers in parenthesis represent one standard deviation.

For sustainable degradation of recalcitrant explosives, such as RDX and HMX, it is necessary to deplete DO to concentrations approaching 0.0 mg/L and to maintain unadjusted ORP values below -250 mV. In earlier treatability studies, Behrends et al[Ref. 2] reported a significant correlation between redox potential and RDX removal (r = .89), indicating that approximately 80% of the variation in RDX removal could be accounted for by variation in redox potential ($R^2 = 0.79$). This may help to explain the variability in treatment efficacy reported in Test III in which ORP values for MRS treatments were greater than -250 mV (Figure 4-1 and Table 4-7).

Values for pH ranged from 6.5 to 7.0 and were very stable during all of the tests. In wetland treatment systems, biological factors such as microbial respiration and degradation of organic matter give rise to weak carbonic acid and organic acids, respectively, which tend to depress pH below neutral (pH 7).

Temperatures were also relatively stable, ranging from 21.9 to 26°C. Minor differences in temperature among treatments may have been influenced by HRT and treatment-mediated differences in evapotranspiration.

EC ranged from 514 to 1,204 µohm/cm and was influenced by carbon substrate, sample location, and date. Relatively large spatial and temporal differences in EC within and among treatments were probably influenced by factors such as substrate solubility, loading criteria (daily versus biweekly), and inherent differences in total dissolved solids content of the carbon substrates.

4.5 Nutrient Dynamics

Nutrient dynamics in treatment systems receiving MRS, molasses syrup, or sewage slurry are summarized in Tables 4-8, 4-9, and 4-10. Ammonium (NH_4) dynamics were consistent with changes expected under conditions of rapid anaerobic development. Temporal and spatial changes in ammonium nitrogen (NH_4-N) concentrations are due to bacterial mineralization (release) and assimilation (uptake), adsorption to the rocks (cation exchange), plant uptake, and nitrification. For example, results from the first sampling of the test conducted with MRS

Table 4-8

Nutrient Dynamics Data as a Function of Sample Date, Treatment, and Sample Location For Test I - Data for July 1 and July 20, 1997

Date	Treatment	Milk Replacement Starter			Molasses Syrup		
	Parameter (mg/L)	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]
7/1/97	NH4-N	0.2	15.3 (3.52)	7.7 (4.57)	0.2	10.1 (0.07)	10.1 (8.03)
7/1/97	NO3-N	28.6	0.1 (0.0)	0.1 (0.03)	28.6	0.5 (0.27)	0.1 (0.00)
7/1/97	PO4	0.02	0.4 (0.0)	0.3 (0.21)	0.02	<0.01 (0.0)	0.3 (0.00)
7/1/97	TKN	0.64	16.8 (4.22)	9.1 (4.93)	0.64	11.6 (0.21)	11.3 (8.16)
7/1/97	NPOC	4.47	56.3 (25.24)	29.5 (13.04)	4.47	145.2 (35.71)	36.8 (22.91)
7/2/97	COD	10	169 (92.6)	67 (29.0)	10	425 (93.3)	91 (60.8)
Date	Treatment	Milk Replacement Starter			Molasses Syrup		
	Parameter (mg/L)	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]
7/20/97	NH4-N	0.23	9.7 (2.7)	12.4 (1.77)	0.23	2.4 (1.59)	3.9 (0.52)
7/20/97	NO3-N	27.2	0.1 (0.0)	0.1 (0.02)	27.2	0.6 (0.64)	1.1 (0.00)
7/20/97	PO4	<0.01	<0.01(0.0)	<0.01(0.0)	<0.01	<0.01(0.0)	<0.01(0.0)
7/20/97	TKN	0.37	14.1(3.39)	15.8(2.47)	0.37	5.8 (1.41)	6.5(0.57)
7/20/97	NPOC	4	7.0(0.49)	32.3(4.95)	4	49.1(32.88)	56. (15.63)
7/20/97	COD	8	65 (9.90)	37 (9.8)	8	187 (81.3)	148 (96.9)

1) Each value is based on the average of two observations (n=2).
2) Numbers in parenthesis represent one standard deviation.

Table 4-9

Nutrient Dynamics Data as a Function of Sample Date, Treatment, and Sample Location For Test II - Data for August 5 and August 19, 1997

Date	Treatment	Milk Replacement Starter			Sewage Slurry		
	Parameter (mg/L)	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]	Influent (n=1)	Midpoint[1,2]	Effluent[1,2]
8/5/97	NH4-N	0.25	5.8 (1.65)	6.2 (0.99)	0.25	3.1 (0.37)	3.0 (0.59)
8/5/97	NO3-N	27.7	0.2 (0.04)	0.2 (0.00)	27.7	0.2 (0.01)	0.1 (0.06)
8/5/97	PO4	<0.10	0.4 (0.06)	0.3 (0.07)	<0.10	0.6 (0.12)	0.3 (0.35)
8/5/97	TKN	2.14	9.0 (2.07)	8.9 (1.08)	2.14	5.9 (0.40)	6.8 (1.34)
8/5/97	NPOC	3.9	19.1 (5.37	4.9 (0.28)	3.9	14.1 (4.24)	10.9 (0.78)
8/5/97	COD	10	67.5 (10.61)	27.0 (4.24)	10	46.5 (26.16)	36.5 (3.54)
Date	**Treatment**	**Milk Replacement Starter**			**Sewage Slurry**		
	Parameter (mg/L)	**Influent (n=1)**	**Midpoint[1,2]**	**Effluent[1,2]**	**Influent (n=1)**	**Midpoint[1,2]**	**Effluent[1,2]**
8/19/97	NH4-N	0.24	5.3 (3.11)	4.0 (2.19)	0.24	2.8 (0.01)	1.5 (0.01)
8/19/97	NO3-N	26.7	0.1 (0.03)	0.2 (0.01)	26.7	0.2 (2.2)	0.1 (0.00)
8/19/97	PO4	<0.01	0.0 (0.01)	0.4 (0.11)	<0.01	0.3 (0.10)	0.1 (0.00)
8/19/97	TKN	0.48	7.5 (3.37)	5.4 (2.35)	0.48	6.2 (0.05)	5.1 (0.13)
8/19/97	NPOC	4	3.8 (0.57)	5.1 (0.78)	4	13.3 (0.28)	15.4 (0.00)
8/19/97	COD	17	19.5 (3.54)	7.5 (9.19)	17	42.5 (2.12)	44.0 (4.24)

1) Each value is based on the average of two observations (n=2).
2) Numbers in parenthesis represent one standard deviation.

Table 4-10

Nutrient Dynamics Data as a Function of Sample Date, Treatment, and Sample Location For Test III - Data for September 2 and September 16, 1997[1]

Date	Treatment	MRS 7.5			MRS 3.75		Syrup 7.5		Syrup 3.75	
	Parameter (mg/L)	Influent	Midpoint	Effluent	Midpoint	Effluent	Midpoint	Effluent	Midpoint	Effluent
9/2/97	NH4-N	0.16	1.7	1.0	0.14	<0.02	0.14	<0.02	0.39	0.28
9/2/97	NO3-N	26.2	0.04	0.1	0.15	0.08	0.15	0.08	0.17	0.12
9/2/97	PO4	<0.01	0.2	0.1	<0.01	<0.01	<0.01	<0.01	0.4	0.07
9/2/97	TKN	1.1	2.7	2.1	1.97	1.88	1.97	1.88	2.4	1.94
9/2/97	NPOC	4	4.8	4.8	13.5	5.8	13.5	5.8	29.4	6.4
9/3/97	COD	1	11	11	8	5	41.0	100	16	15
Date	Treatment	MRS 7.5			MRS 3.75		Syrup 7.5		Syrup 3.75	
	Parameter (mg/L)	Influent	Midpoint	Effluent	Midpoint	Effluent	Midpoint	Effluent	Midpoint	Effluent
9/16/97	NH4-N	0.13	1.6	1.1	2.25	1.65	<0.02	<0.02	0.17	0.14
9/16/97	NO3-N	25.5	0.09	0.1	3.2	0.14	0.21	0.04	0.18	0.19
9/16/97	PO4	<0.01	0.2	0.1	0.19	0.36	0.02	0.02	0.66	0.55
9/16/97	TKN	0.5	2.7	1.9	3.99	3.74	1.28	1.34	3.49	3.06
9/16/97	NPOC	4.3	4.2	5.2	3.2	4	40	70.9	18.9	4.2
9/17/97	COD	8	11	55	14	28	99.0	73	36	43

1) All values are based on individual observations (n=1).

on July 1, 1997, (Test I, see Table 4-8), revealed low NH_4 concentrations in the influent groundwater (0.2 mg/L), but relatively high NH_4 concentrations at the midpoint (15.3 mg/L), with significantly less in the effluent (7.7 mg/L). This is consistent with rapid mineralization of organic nitrogen contained in the MRS powder, followed by adsorption and rapid assimilation of the ammonia by plants and a rapidly growing microbial population. However, adsorption and plant/microbial assimilation are short-lived phenomena. Sikora et al. (1994)[Ref. 8] demonstrated that cation exchange sites on river gravel are limited. Furthermore, assimilation by plants and microbes are limited by growth rate and ultimately by biomass. Microbial nitrification is insignificant in anaerobic environments, as free oxygen in excess of 2 mg/L is required for the process.

By the second sampling date (July 20, 1997), NH_4 concentrations in test cells containing MRS were increasing from the midpoint to the effluent (9.7 versus 12.4 mg/L), again consistent with rapid mineralization of organic nitrogen, but with reduced microbial/plant assimilation, adsorption, and nitrification.

Ammonium concentrations in the test cells amended with molasses syrup and sewage slurry (Test II) were significantly less than those observed in test cells amended with MRS (Tables 4-8, 4-9, and 4-10). These differences were due to inherent differences in the amount of organic nitrogen in the respective amendments and the fact that the ammonium was supplied to the test cells containing molasses syrup as a batch-loaded inorganic fertilizer (e.g., monoammonium phosphate). Furthermore, differences in NH_4 concentration between similar treatments with MRS (Tests I, II, and MRS 7.5) can be explained by differences in wetland maturation and sampling error due to spatial and temporal variability.

Influent NH_4 concentrations were low (0.13 to 0.25 mg/L) during all of the tests; however, mid-point data indicate a general increase in NH_4 concentrations (Tables 4-8, 4-9, and 4-10). This suggests that NH_4 was being produced within the cells. Generally, the NH_4 concentrations drop prior to being discharged. Reduction in NH_4 concentrations from midpoint to effluent ranged from -12 to 100% and averaged 29% (Figure 4-2).

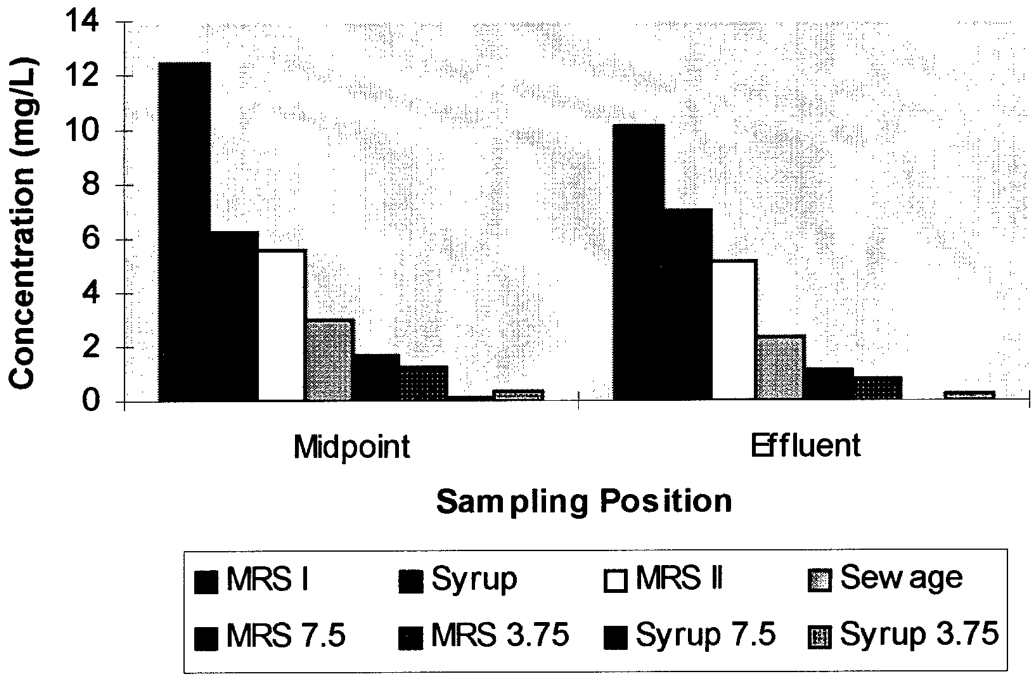

Figure 4-2

Ammonium Concentration (mg/L) as a Function of Sampling Position and Treatment[1]

1) Tests I and II values are based on averages of two replicates over two sampling periods (n=4) during summer months. Test III values are based on averages of one value over two sampling periods (n=2).

TKN is a measure of organic nitrogen and ammonia. Since ammonium is a major nutrient in organically and inorganically fertilized wetland systems, TKN dynamics generally follow the same trends as ammonium (see table values). In more mature wetland systems, the ratio of ammonia to organic-N changes to reflect a larger contribution by the organic fraction.

Nitrate concentrations in the groundwater were relatively high, ranging from 26-29 mg/L. However, under the anoxic/anaerobic conditions existing within the test cells and with ample supplies of organic carbon, the nitrate was rapidly reduced by denitrifying bacteria to concentrations near 0.1 mg/L (>99.5 % removal).

Phosphorus dynamics in constructed wetlands are very complex and are influenced by mineralization of organic matter, plant uptake (seasonal), adsorption, and precipitation.[Ref. 9] The initial rapid removal of phosphorus in this test (P concentration <0.01 mg/L during second sampling) was probably influenced by rapid plant uptake, sorption, and precipitation with iron, aluminum, and calcium minerals.[Ref. 8] However, sorption sites in most gravel-based systems are considered limited and, thus, provide only marginal removal and only during the initial stages of wetland operation. This is consistent with results of Tests II and III in which phosphorus concentrations were elevated in the effluent, relative to effluent concentrations observed in Test I.

NPOC and COD are both measures of potential oxygen demand and are useful for monitoring degradation of labile organic substrates such as MRS, molasses syrup, and sewage slurry. Their respective dynamics are influenced by availability of free oxygen, rate and frequency of fertilization, and hydraulic retention time. COD and NPOC removal rates during Tests I and II were highly variable, ranging from 78% removal of molasses syrup COD on July 2, 1997, to less than 21% removal of molasses syrup COD on July 20, 1997. Such high levels of treatment variability are suspected to be due to large spatial and temporal differences in organic carbon concentration within a test cell resulting from batch loading, plug flow hydraulics, and differential flow paths (i.e., short circuiting).

SECTION 5.0

SUMMARY AND CONCLUSIONS

Degradation and removal of explosives in gravel-based test systems were influenced by organic carbon source (MRS versus molasses syrup versus sewage slurry) method of application (daily versus biweekly) and hydraulic retention time (7.5 an 3.75 days). The primary explosives TNT, RDX, and HMX, and the respective by-products of TNT and RDX, were often rapidly reduced to below method detection limits. However, in certain instances, residual concentrations of RDX, HMX, and by-products of TNT and RDX persisted. Analysis of data indicated that in certain instances, lack of removal was correlated with either short hydraulic retention times (HRT = 3.75 days) and/or elevated redox potential (ORP > -250). Treatment efficacy of the various organic carbon sources may have also been influenced by carbon substrate solubility and method of application (batch loading on a biweekly basis versus incremental loading on a daily basis). Preliminary results indicated that daily application of a relatively soluble organic substrate, such as molasses syrup, provided better explosives removal than less soluble substrates which were batch loaded at two-week intervals (MRS and sewage slurry).

Based on the fact that molasses syrup is an order of magnitude less costly than MRS, is more soluble and, thus, easier to apply, and provides enhanced explosives removal, it is recommended that molasses syrup replace MRS as the standard carbon substrate for anaerobic wetlands for treating explosives-contaminated water. Where feasible, it may also be advantageous to utilize other sources of inexpensive organic carbon, such as liquefied municipal sewage and/or liquefied animal manures.

With respect to hydraulic retention time, it is recommended that the standard HRT of 7.5 days be maintained since residual explosive by-products were evident in the effluent of treatments with 3.75 HRT.

SECTION 6.0

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APPENDIX A

METHODS AND PROCEDURES

APPENDIX A-1

Procedure for Explosives: Method AP-0062

Tennessee Valley Authority

Specialty Laboratory of Land and Water Sciences and Remediation
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : AP-0062

Title: Extraction, Preparation, and Analysis of Explosives and Their Degradation Products

Signature	Title	Date
Prepared by: W. David Phillips W. David Phillips	Chemist	4/14/98
Concurred: Eugene A. Zarate Eugene A. Zarate	Laboratory Section Leader	4/14/98
Concurred: William J. Rogers William J. Rogers	QA Officer	4/13/98
Concurred:		
Approved: Kathy [illegible] for Joe Hoagland Joseph J. Hoagland	Manager	4/16/98

Revision	R0	R1				
Control Date	17-Oct-97	13-Apr-98				

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1.0 PURPOSE

This procedure is a method of determination for the identification and quantitation of nitroaromatics and nitroamines using High Performance Liquid Chromatography (HPLC).

2.0 SCOPE

This procedure applies to water, compost leachate, soil, sediment, gravel, and plant samples. The following analytes (listed with their abbreviations as used in this document) can be identified and quantified with this procedure.

2,6-Diamino-4-nitrotoluene----------------------------2,6-DANT
1,3,5-Trinitroso-1,3,5-triazacyclohexane--------------Tri-RDX
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine-----HMX
2,4-Diamino-6-nitrotoluene----------------------------2,4-DANT
1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane--------Mono-RDX
Hexahydro-1,3,5-trinitro-1,3,5-triazine----------------RDX
1,3,5-Trinitrobenzene------------------------------------TNB
1,3-Dinitrobenzene--1,3-DNB
3,5-Dinitroaniline---3,5-DNA
2,4,6-Trinitrotoluene--------------------------------------TNT
2-Amino-4,6-dinitrotoluene-----------------------------2-ADNT
4-Amino-2,6-dinitrotoluene-----------------------------4-ADNT
2,6-Dinitrotoluene--2,6-DNT
2,4-Dinitrotoluene--2,4-DNT
4,4',6,6'-Tetranitro-2,2'-azoxytoluene-----------------TN-2,2'-AZT
2,4',6,6'-Tetranitro-2',4-azoxytoluene-----------------TN-2',4-AZT
2,2',6,6',Tetranitro-4,4'-azoxytoluene-----------------TN-4,4'-AZT
2,2'-Dinitro-4,4'-azoxytoluene--------------------------DN-4,4'-AZT

3.0 SUMMARY

Samples can be prepared for analysis with no prior extraction or concentration, or can be extracted and concentrated before analysis preparation. Sample extraction and concentration methods may also serve to remove substances which would interfere with analyte identification or quantitation. The resulting prepared sample is injection ready for HPLC analysis. Nitroaromatics and nitroamines in the prepared sample are chromatographically separated as they pass through a HPLC analytical column. The nitroaromatic and nitroamine compounds are identified by

comparing their retention times and UV spectra, generated on a photodiode array detector using commercial chromatography workstation software, with those of known standard compounds generated under similar conditions. The compounds are quantified by comparing their peak heights, generated on a single wavelength UV/VIS detector, with compound-specific calibration curves generated under identical conditions.

4.0 REFERENCES

4.1 "Method 8330 - Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)." EPA Test Methods for Evaluating Solid Waste (SW-846), November 1992

4.2 Personal communications with Dr. Thomas Jenkins
U.S. Army Cold Regions Research and Engineering Laboratory
Hanover, NH

4.3 Personal communications with Philip G. Thorne
U.S. Army Cold Regions Research and Engineering Laboratory
Hanover, NH

4.4 Thorne, Philip G. "Hydrolytic Release of Bound Residues From Composted TNT-Contaminated Soil." 1996

4.5 Personal communications with Dr. Steve Larson
U.S. Army Corps of Engineers Waterways Experiment Station, Environmental Laboratory, Environmental Chemistry Branch

4.6 GLP-0018, "Method Detection Limits", Environmental Applications, Tennessee Valley Authority, Muscle Shoals, Al

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the Supervisor of the Environmental Applications section, or his designee, to ensure that this procedure is followed during the handling, preparation, extraction and analysis of all samples for nitroaromatics and nitroamines by HPLC.

5.2 The Laboratory Group Leader, or his designee, shall delegate the performance of this procedure to personnel experienced with this procedure. Training of

personnel inexperienced with this procedure shall be carried out by experienced personnel under the supervision of the Laboratory Group Leader.

5.3 The analyst shall follow this procedure and report any abnormal results or problems to the Laboratory Group Leader, or his designee.

6.0 REQUIREMENTS

6.1 Prerequisites

Method detection limits shall be determined as in GLP0018 (see Note 9.1)

6.2 Limitations and Actions

None

6.3. Materials/Apparatus/Equipment

6.3.1 HPLC system composed of a tertiary pump (Varian Model 9012 or equivalent), an autosampler (Varian Model 9300 or equivalent) and a single wavelength UV/Vis detector (Varian Model 9050 or equivalent) or a photodiode array detector (Varian Model 9065 or equivalent).

6.3.2 HPLC guard column - Ultracarb ODS (20), 30 X 4.6 mm, manufactured by Phenomenex - (or equivalent).

6.3.3 HPLC analytical column - Ultracarb ODS (20), 250 X 4.6 mm, manufactured by Phenomenex - (or equivalent).

6.3.4 Tissue homogenizer - Omni Mixer ES, manufactured by Omni International (or equivalent).

6.3.5 25 mm sawtooth generator probe for use with tissue homogenizer - Part # 15035, manufactured by Omni International (or equivalent).

6.3.6 Freeze Dryer - Model 77520 (6L-Benchtop), manufactured by Labconco - (or equivalent).

6.3.7 Sonicator bath - Bransonic 52, manufactured by Bransom of Smith/Kline (or equivalent).

6.3.8 Temperature controlled circulating bath - Model 2095 Bath and Circulator, manufactured by Forma Scientific - (or equivalent).

6.3.9 300 ml size freeze dry flask with rubber top and glass adapter - Assembly # 75406, manufactured by Labconco - (or equivalent).

6.3.10 Glass Class A volumetric pipets (various sizes).

6.3.11 Glass graduated cylinders (various sizes).

6.3.12 Glass Class A volumetric flasks (various sizes).

6.3.13 Glass separatory funnels 125 ml and 250 ml size.

6.3.14 Stainless steel spatulas.

6.3.15 Teflon coated stir bars (various sizes).

6.3.16 Heavy duty aluminum foil - Part # 0-10900, manufactured by Reynolds Aluminum Co. - (or equivalent).

6.3.17 12-port vacuum manifold - Cat. # 5-7030, manufactured by Supelco Inc - (or equivalent).

6.3.18 Sep-Pak Vac Adapters - Part # WAT054260, manufactured by Waters Corp. - (or equivalent).

6.3.19 60 ml Sep-Pak reservoir - Part # WAT024659, manufactured by Waters Corp. - (or equivalent).

6.3.20 Explosion-proof refrigerator - Model Cryo-Fridge, manufactured by Scientific Products Inc. - (or equivalent).

6.3.21 8ml and 16 ml glass vials with Teflon lined closures - Cat # 75008-SB and 75016-SB respectively, manufactured by Scientific Resources Inc. (SRI) - (or equivalent).

6.3.22 12 X 32 amber autosampler vials with Teflon lined closures - Cat. # 99575-A - (or equivalent).

6.3.23 250 ml tall form, wide mouth glass bottle with Teflon lined closures - Part # 131-08C/TL/WS, manufactured by Eagle Picher - (or equivalent).

6.3.24 60 ml pre-cleaned amber bottle with Teflon lined closure - Part # 120-02A, manufactured by Eagle Picher - (or equivalent).

6.3.25 40 ml vial with Teflon lined closure - Part # 141-40A, manufactured by Eagle Picher - (or equivalent).

6.3.26 10 ml disposable plastic syringe - Part # 309604, manufactured by Becton and Dickinson - (or equivalent).

6.3.27 25 mm, PTFE syringe filters having 0.2 or 0.45 µm pore size - Cat. # 42225-NP and 44525-PC respectively, manufactured by SRI - (or equivalent).

6.3.28 Alumina-A solid phase extraction cartridges 1, 5, and 10 gram sizes - Part # WAT054580, WAT054670 and WAT054710 respectively, manufactured by Waters Corp. - (or equivalent).

6.3.29 Porapak-Rdx solid phase extraction cartridge (500mg size) - Part # WAT047220, manufactured by Waters Corp. - (or equivalent).

6.3.30 Vacuum manifold for solid phase extraction cartridges - Cat. # 5-7030, manufactured by Supelco Inc. - (or equivalent).

6.3.31 Refrigerated centrifuge - Model CRU-5000, manufactured by IEC Inc. - (or equivalent).

6.3.32 Benchtop centrifuge - Model SS-4 Manual, manufactured by Sorvall - (or equivalent).

6.3.33 Magnetic stirrer - Cat. # 14-511-1A, manufactured by Fisher Scientific Co. - (or equivalent).

6.3.34 Analytical balance - Model A200S, manufactured by Sartorius - (or equivalent).

6.3.35 Glass vacuum desiccator with indicating desiccant.

6.3.36 Ceramic mortar and pestle..

6.3.37 Glass conical bottom centrifuge tubes (12 ml size).

6.3.38 30 mesh sieve.

6.3.39 Pasteur pipets - Cat. # P5201-1, manufactured by Scientific Products - (or equivalent).

6.3.40 Parafilm “M” - Laboratory Film, manufactured by American National Can (or equivalent).

6.3.41 Ultrapure nitrogen - compressed gas.

6.4 Reagents and Standards

6.4.1 Water (HPLC grade) - Part # WX0004-1, manufactured by E M Science - (or equivalent).

6.4.2 Methanol, CH_3OH (HPLC grade) - Part # MX0488-1, manufactured by E M Science - (or equivalent).

6.4.3 Acetonitrile, CH_3CN (HPLC grade) - Part # AX0142-1, manufactured by E M Science - (or equivalent).

6.4.4 Sodium chloride, NaCl (reagent grade) - Part # SX0420-1, manufactured by E M Science - (or equivalent).

6.4.5 Calcium chloride, $CaCl_2$ (reagent grade) - Part # C1096, manufactured by Spectrum Chemical - (or equivalent).

6.4.6 Sodium phosphate dibasic, $Na_2HPO_4 \cdot 7H_2O$ (reagent grade) - Part # SX0175-1, manufactured by E M Science - (or equivalent).

6.4.7 Concentrated sulfuric acid, H_2SO_4 (reagent grade) - Part # 5557, manufactured by Mallinckrodt Inc. - (or equivalent).

6.4.8 Blank soil - U.S. Army Environmental Center Standard Soil.

6.4.9 Neat explosive analyte standards - either provided by the U.S. Army Environmental Center, or purchased from Accustandard Inc, Stanford Research Institute International or Chem Service Inc.

6.4.9.1 Stock Standard Solutions (single analyte)

Each neat solid analyte standard is dried to a constant weight in a vacuum dessicator at room temperature in the dark. Each neat liquid analyte standard is transferred using glass Pasteur pipets or glass gas-tight syringes with Teflon tipped plungers. Approximately 0.1 g (weighed to 0.0001 g) of a single neat analyte is placed into a 100 ml volumetric flask and diluted to volume with acetonitrile. A stir bar is added to the flask which is then placed on a magnetic stirrer and swirled until the analyte has totally dissolved or mixed. During mixing, the flask is covered with an aluminum foil hood. The stir bar is removed, the flask is stoppered and wrapped in aluminum foil. The concentration of the stock solution is calculated from the actual weight of the analyte used, the purity of the analyte and the volume of the solution (nominal concentration is 1,000 mg/L). These solutions should be stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4° C.

6.4.9.2 Intermediate Standard Solutions (single or multiple analyte)

These solutions, at approximately 20 µg/ml per analyte, are prepared by dilutions of the stock standard solutions with acetonitrile in volumetric flasks. The flasks are wrapped with aluminum foil and stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4° C. These solutions are used to prepare calibration standards.

6.4.9.3 Calibration Standard Solutions (multiple analyte)

These solutions, at a minimum of five levels covering the concentration range of interest (approximately 6 µg/ml to approximately 25 ng/ml), are usually prepared by dilutions of the Intermediate standard solutions with acetonitrile in volumetric flasks. The flasks are wrapped with aluminum foil and stored, stoppered and sealed with Parafilm, in an explosion-proof refrigerator at 4°C. Before analysis, these solutions are equilibrated to room temperature, diluted 1:1 with water, allowed to stand for 20 minutes and passed through a 25 mm PTFE syringe filter with 0.45 µm pore size.

6.4.10 Aqueous spike solution (8 component) at 2 µg/ml each analyte for use with water, soil, and sediment.

Spike made from Accustandard solution containing HMX, RDX, (1,3,5-TNB), (1,3-DNB), nitrobenzene, TNT, 2-ADNT and 2,4-DNT at 1000

μg/ml each analyte. The spike solution is prepared by first diluting 1.0 ml of the 1000 μg/ml standard to 25.0 ml with acetonitrile. A 5.0 ml aliquot of this solution is diluted to 100.0 ml with HPLC water to yield a solution whose concentration is 2.0 ug/ml per analyte.

6.4.11 Spike solution for use with compost leachate extraction

Use the calibration standard solution(s) whose concentration is approximately 3 ug/ml per analyte for the following compounds: (2,6-DANT), HMX, (2,4-DANT), RDX, (1,3,5-TNB), TNT, 4-ADNT, 2-ADNT, (2,6-DNT) and 2,4-DNT.

6.4.12 Spike solution for use with plant extraction

Prepare a spiking solution at approximately 100 ug/ml per analyte for the following analytes: (2,6-DANT), HMX, (2,4-DANT), RDX, (1,3,5-TNB), TNT, 4-ADNT, 2-ADNT, (2,6-DNT) and 2,4-DNT. The mixed analyte solution matrix should be acetonitrile. The solution should be prepared from stock standard solutions (section 6.4.9.1) and should be stored in an aluminum foil wrapped flask, stoppered and sealed with Parafilm in an explosion-proof refrigerator at 4° C.

6.4.13 Sulfuric acid solution (1+1)

In a suitable container place 50.0 ml of HPLC water, add to this slowly and with swirling 50.0 ml of concentrated sulfuric acid. Allow to equilibrate to room temperature before using.

6.4.14 Sodium phosphate dibasic at 1.07 M concentration

Place appropriate quantity of sodium phosphate dibasic heptahydrate in a glass beaker and place in a forced air oven at 35°C and leave for at least 24 hr. Remove from oven and allow to cool. For 1 L of solution, weigh out 322 g of the dried compound and place in a large Erlenmeyer flask. Add 1000 mL of HPLC water to the flask. Add a stir bar and place on a magnetic stirrer / hot plate on low heat and moderate stirring until all solids are dissolved. Cool to room temperature before use. This should not be kept for use for more than 2 days.

6.4.15 Aqueous sodium chloride solution

Weigh out 325 g of sodium chloride and place in a 1000 ml volumetric flask. Make flask to volume with HPLC water. Add a stir bar and place on magnetic stirrer until the solution is saturated (usually 30 minutes).

6.4.16 Aqueous calcium chloride solution

Weigh out 6.67 g of calcium chloride dihydrate and place in a 1000 ml volumetric flask. Make to volume with HPLC water, add a stir bar and place on a magnetic stirrer until all solids are dissolved.

6.5 Quality Control Sample Requirements

6.5.1 Every batch of samples (20 members or less) whose matrix is water, soil, sediment, or gravel, shall have the following QA/QC samples extracted and/or prepared at the same time in identical fashion: matrix spike, matrix spike duplicate, method blank and laboratory control sample (LCS).

Every batch of samples (20 members or less) whose matrix is compost leachate shall have the following QA/QC samples extracted at the same time and in identical fashion: matrix spike, method blank and LCS.

Every batch of samples (20 members or less) whose matrix is plant tissue shall have the following QA/QC samples extracted at the same time and in identical fashion: matrix spike

6.5.2 Daily Calibration Check of the UV/VIS detector system.

Midpoint calibration standards for each analyte of interest are analyzed in duplicate at the beginning of the analytical run, singly after every 10 sample vials and singly after the last sample of the run. The calculated concentration of each analyte of interest in each midpoint standard throughout the analytical run shall agree with its known value within +/- 15%. If this criterion is not met, samples following the previous acceptable standard and prior to the next acceptable standard may be reanalyzed, or all or part of the sample data may be "qualified" and flagged with a "Q" designation in the database. The decision to reanalyze or qualify samples shall be made by the Laboratory Group Leader.

7.0 PROCEDURE

7.1 Calibration

Initial Calibration of the UV/VIS detector system.

From one to three injections of each calibration standard over the concentration range of interest are sequentially injected into the HPLC in random order. Using commercial chromatography software, peak heights are obtained for each analyte. Calibration curves are generated using spreadsheets which utilize linear regression equations of the form $y = mx$, $y = a + bx$, or $y = a + bx + cx^2$. Selection of the equation form to use is made by assessing the data for goodness of fit and how closely back-calculation of the fit data reproduces the known concentrations of the calibration solutions.

7.2 Procedure Instructions

NOTE: Because some of the analytes of interest in the following procedures are photosensitive and thermolabile, standards, samples, extracts, filtrates, eluants, etc. should be exposed to light or heat as little as possible during the performance of the procedures. This is especially true during standing or storage periods.

7.2.1 Preparation of water samples (with no preconcentration) for qualitative / quantitative analysis.

NOTE: Batches of samples undergoing this preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.1.1 Retrieve samples and allow them to equilibrate to room temperature if necessary.

7.2.1.2 Obtain the appropriate sample worksheet (Attachment 1 - "Preparation of Liquids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.1.3 Place appropriate volume of sample in a properly labeled, 40 mL vial and seal with a Teflon-lined closure.

7.2.1.4 Centrifuge the sample at 2000 rpm or greater for 30 minutes.

7.2.1.5 Decant the supernatant to a properly labeled, glass vial (40 ml size) and seal with Teflon-lined closure.

7.2.1.6 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - To a 10 ml volumetric flask, add 5.0 ml of selected sample, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make flask to volume with HPLC water, stopper and mix thoroughly by inversion. Wrap flask with aluminum foil.

Laboratory control sample - To a 10 ml volumetric flask, add 5.0 ml of HPLC water, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make flask to volume with HPLC water, stopper and mix thoroughly by inversion. Wrap flask with aluminum foil.

Method blank - To a 10 ml volumetric flask, add 10.0 ml of HPLC water, stopper the flask and mix thoroughly by inversion.

7.2.1.7 Using precisely measured volumes, place equal amounts of sample (regular and QA/QC) and HPLC grade acetonitrile (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.1.8 Let mixture stand in dark at room temperature for 20 minutes.

7.2.1.9 Pass the mixture through a PTFE syringe filter with 0.45 μm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and

quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.1.10 Let the filtrate stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto the autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.1.11 If it becomes necessary during the analysis process to dilute the sample, record on the sample worksheet the aliquots and dilution volumes used.

7.2.2 **Preconcentration of water samples by Solid Phase Extraction (SPE) for qualitative/quantitative analysis by HPLC.**

NOTE: Batches of samples undergoing this extraction shall contain the following QA/QC samples: LCS and method blank

7.2.2.1 Retrieve the samples and allow them to equilibrate to room temperature.

7.2.2.2 Obtain the appropriate sample worksheet (Attachment 2 - "Preconcentration of Liquids by SPE for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be concentrated and prepared for HPLC analysis, the date, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

7.2.2.3 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Laboratory control sample - To a 50 ml volumetric flask, add approximately 25 ml of HPLC water, then add 1.0 ml of the aqueous spiking solution (see section 6.4.10). Make the flask to volume with HPLC water, stopper and mix thoroughly by inversion. Let stand in dark until needed.

Method blank - To a 50 ml volumetric flask, add 50 ml of HPLC water. Stopper and mix thoroughly by inversion. Place in dark until needed.

7.2.2.4 Fractionate an appropriate volume of sample to 40 ml vials with Teflon lined closures and centrifuge for 30 minutes at 2000 rpm or greater.

7.2.2.5 Remove the supernatant and place in an Erlenmeyer flask, seal with Parafilm, and let stand in dark until needed. This supernatant is the sample fraction that will be concentrated by SPE.

NOTE: Do not allow the resin bed of the Porapak-Rdx cartridge to become dry during the conditioning step, between the conditioning and the sample loading steps or during the sample loading.

7.2.2.6 Attach an adapter and a 60 ml reservoir to a Porapak-Rdx cartridge and connect the cartridge to the vacuum manifold. Condition the cartridge by passing 15 ml of acetonitrile through it (drip by gravity), followed by 30 ml of water at a rate of about 10 ml/minute using vacuum.

NOTE: In the following step, if the extract from this procedure is to be used for analyte identification only, approximately 60 ml of sample is used. If the extract is to be used for analyte quantitation, it is necessary to use a known volume aliquoted with a graduated cylinder (rinse cylinder three times with HPLC water and add this to reservoir for the appropriate sample).

7.2.2.7 Pass the appropriate volume of sample through the SPE cartridge at a flow rate of about 10 ml/min. Record this known or approximate volume in the appropriate area on the worksheet.

7.2.2.8 After the sample has been totally pulled through the cartridge, continue to apply a vacuum to the cartridge for about 5 minutes to remove residual water.

7.2.2.9 Remove the SPE cartridge from the vacuum manifold and remove the adapter and 60 ml reservoir from the cartridge.

7.2.2.10 Position the SPE cartridge over a properly labeled 5 ml volumetric flask (other sizes of volumetric flasks can be used if deemed necessary) with the Luer tip of the cartridge extending into the mouth of the flask.

7.2.2.11 Add 5.0 ml of acetonitrile to the SPE cartridge.

7.2.2.12 Apply a lightly pressurized flow of ultrapure nitrogen to the top of the cartridge in order to initiate solvent flow through the cartridge and into the volumetric flask. Once the solvent flow begins, remove the nitrogen source and allow the solvent flow to be by gravity alone.

7.2.2.13 After the solvent stops dripping from the cartridge, reapply the pressurized nitrogen to the top of the cartridge to force any trapped solvent into the flask.

7.2.2.14 Make the flask to volume with acetonitrile, stopper and mix thoroughly. Record the flask volume in the appropriate area on the worksheet.

7.2.2.15 Using precisely measured volumes, place equal amounts of eluant and HPLC grade water (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.2.16 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.2.17 Pass the mixture through a PTFE syringe filter with 0.45 μm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.2.18 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.3 **Preparation of soil or sediment samples for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.3.1 Spread enough sample either onto acetonitrile rinsed ceramic dishes or clean aluminum foil for sample duplicates, matrix spikes, matrix spike duplicates, and percent moisture procedure.

7.2.3.2 Place the samples in the air flow of a darkened hood at room temperature and allow to dry for 12 - 18 hours (no visible moisture should be present).

7.2.3.3 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

Set aside enough air dried sample, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.3.4 Grind an appropriate quantity of air dried sample in an acetonitrile rinsed mortar. Pass the ground sample through a clean 30 mesh sieve onto clean aluminum foil. Place the sieved sample into a glass vial with Teflon-lined closures. Store the sample in the dark at room temperature until ready for use.

7.2.3.5 Obtain the appropriate sample worksheet (Attachment 3 - "Preparation of Solids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be extracted and prepared for HPLC analysis, the date, your name, the sample matrix, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

7.2.3.6 Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the laboratory number of this sample in the appropriate areas in the QC section of the sample worksheet.

7.2.3.7 Into a properly labeled 16 ml glass vial, weigh out 2 grams of air dried, ground and sieved sample. Record the sample weight to the nearest 0.0001 g on the worksheet in the appropriate area.

7.2.3.8 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - For each spiked sample, weigh into a properly labeled 16 ml glass vial, 2 grams of air dried, ground and sieved sample. Record the weight to the nearest 0.0001 g. Add 1.0 ml of the aqueous spiking solution (see section 6.4.10) to the sample, recap the vial and let the sample stand in the dark for 1 hour. Then add 9.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

Laboratory control sample - Into a properly labeled 16 ml glass vial, weigh out 2 g of standard soil (see section 6.4.8). Record the weight to the nearest 0.0001 g. Add 1.0 ml of the aqueous spiking solution (see section 6.4.10) to the sample, recap the vial and let the sample stand in the dark for 1 hour. Then add 9.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

Method blank - Into a properly labeled 16 ml glass vial, weigh out 2 g of standard soil (see section 6.4.8). Record the weight to the nearest 0.0001 g. Add 1.0 ml of HPLC water to the sample, recap the vial and let the sample stand in the dark for 1 hour. Add 9.0 ml of acetonitrile to the vial. Recap the vial and vortex for 1 minute. Place vial in the dark until ready for step 7.2.3.10.

7.2.3.9 To all regular samples (non-QA/QC samples), add 10.0 ml of HPLC grade acetonitrile to the vial, replace the Teflon-lined closure, and vortex for 1 minute. Record this volume on the worksheet in the appropriate area. Place vial in the dark until ready for next step.

7.2.3.10 Suspend the extraction vials (regular samples and QA/QC samples) in a sonicator bath regulated between 10° C and 25° C and sonicate under low light conditions for 18 hours. The water level in the sonicator should be above the solvent level in the sample bottles.

7.2.3.11 Remove the vials from the sonicator bath and let stand in the dark at room temperature for 30 - 60 minutes. This allows particulates to settle and a pipetable supernatant to form.

If, at the end of the standing period, there is still a large volume of suspended particulates, it will be necessary to transfer the sample to a 40 ml vial and centrifuge it at 2000 rpm or greater for 30 minutes.

7.2.3.12 With a volumetric pipet, remove an appropriate quantity of supernatant and mix it at 1:1 ratio with the calcium chloride solution (see section 6.4.16). Let the mixture stand in the dark for 20 minutes.

7.2.3.13 Remove the supernatant from the sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 μm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.3.14 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.4 **Preparation of gravel samples for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, matrix spike duplicate, LCS, and method blank.

7.2.4.1 Spread enough sample either onto acetonitrile rinsed ceramic dishes or clean aluminum foil for sample duplicates, matrix spikes, matrix spike duplicates, and percent moisture procedure.

7.2.4.2 Place the samples in the air flow of a darkened hood at room temperature and allow to dry for 12 - 18 hours (no visible moisture should be present).

7.2.4.3 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

Set aside enough air dried sample, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.4.4 Obtain the appropriate sample worksheet (Attachment 3 - "Preparation of Solids for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, your name, the sample matrix type the serial number of the spiking solution to be used, and the concentration of the spiking solution.

Select one sample out of the batch for use in the creation of the matrix spike and matrix spike duplicate. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.4.5 Weigh enough air dried sample into a pre-cleaned, 250 ml, wide-mouth, tall-form bottle to reach the base of the bottle's neck (usually over 200 g). Record this weight on the worksheet in the appropriate area.

7.2.4.6 Prepare the batch QA/QC samples as listed below. Record all critical data in the appropriate areas in the QC section of the sample worksheet.

Matrix spike and matrix spike duplicate - For each spiked sample, weigh out into a properly labeled 250 ml bottle, enough air dried sample to reach the base of the bottle neck and cap the bottle with a Teflon-lined closure. Record the weight to the nearest 0.1 g. Add 5.0 ml of the aqueous spiking solution (see section 6.4.10) to the sample, recap the bottle and let the sample stand in the dark for 1 hour. Then add 95.0 ml of acetonitrile to the vial. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

Laboratory control sample - Into a properly labeled 250 ml bottle, pipet 5.0 ml of the aqueous spiking solution (see section 6.4.10), recap the vial and let the bottle stand in the dark for 1 hour. Then add 95.0 ml of acetonitrile to the bottle. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

Method blank - Into a properly labeled 250 ml bottle, place 100.0 ml of acetonitrile. Recap the bottle tightly and shake vigorously for 1 minute. Place the bottle in the dark until ready for step 7.2.4.9.

7.2.4.7 For any regular sample (non-QA/QC samples) add 100.0 ml of acetonitrile to the bottle and replace the cap, taking care to ensure a tight fit. Record this volume on the worksheet in the appropriate area.

7.2.4.8 Shake the bottle vigorously for one minute.

7.2.4.9 Place bottle in a sonicator bath regulated between 10° C and 25° C and sonicate for 18 hours. The water level in the sonicator should be even with the solvent level in the sample bottles, but should be high enough to float the bottles or touch the lids of the sample bottles.

7.2.4.10 Remove bottle from sonicator bath and shake vigorously for one minute.

7.2.4.11 Let the bottle stand in the dark and equilibrate to room temperature.

7.2.4.12 Mix, in a glass vial, an appropriate volume of the acetonitrile extract at a 1:1 ratio with a calcium chloride solution (see section 6.4.16) and let stand in the dark for 20 minutes.

7.2.4.13 Remove the supernatant from the calcium chloride treated sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 µm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.4.14 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.5 **Preparation of compost leachate for qualitative/quantitative analysis**

NOTE: Batches of samples undergoing this extraction/preparation shall contain the following QA/QC samples: matrix spike, LCS, and method blank.

7.2.5.1 Retrieve the samples and allow to equilibrate to room temperature if necessary.

7.2.5.2 Obtain the appropriate sample worksheet (Attachment 4 - "Preparation of Compost Leachates for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be extracted and prepared for HPLC analysis, the date, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

Select one sample out of the batch for use in the creation of the matrix spike. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.5.3 Fractionate approximately 160 ml of the leachate sample into 40 ml glass vials with Teflon lined closures. Centrifuge the vials for 30 minutes at 2000

rpm or greater. After centrifugation, decant the total supernatant for one sample into a 250 ml Erlenmeyer flask.

7.2.5.4 Prepare a sodium chloride solution as per section 6.4.15 of this procedure.

7.2.5.5 Add 12.56 g of sodium chloride to a 500 ml separatory funnel. Measure out 38 ml of sample (use HPLC water for the method blank and LCS) and transfer to the separatory funnel containing the salt. Record these weights and volumes on the worksheet in the appropriate areas.

7.2.5.6 To the matrix spike and LCS, add 1.0 ml of the appropriate spiking solution (see section 6.4.11). Record the critical data on the worksheet in the appropriate areas.

7.2.5.7 Stopper the separatory funnel and shake until the salt has dissolved (about 5 minutes).

7.2.5.8 Using volumetric pipets, add 9.0 ml of acetonitrile to the separatory funnel of each sample which has been spiked and 10.0 ml of acetonitrile to the funnel of each unspiked sample.

7.2.5.9 Stopper the funnel and shake for 5 minutes, then let the samples stand for 10 minutes to allow phases to separate.

7.2.5.10 Drain off the salt layer (bottom layer) except the last 1-2 ml and discard properly. Drain the acetonitrile layer (top layer of approximately 1-2 ml) along with the remaining salt layer into a 250 ml separatory funnel.

7.2.5.11 Add 16 ml of HPLC grade acetonitrile to first separatory funnel and rinse into second separatory funnel.

7.2.5.12 Add 84 ml (measure with 100 ml graduated cylinder) of salt solution (see step 7.2.5.4) to the second separatory funnel.

7.2.5.13 Shake the separatory funnel for 5 minutes and allow to stand for 10 minutes for phase separation.

7.2.5.14 Discard to waste most of the bottom layer (salt) and transfer the acetonitrile layer (top layer) plus the last 1-2 ml of the salt layer to a glass centrifuge tube. Rinse the separatory funnel with 1.0 ml of acetonitrile and transfer to the same centrifuge tube.

7.2.5.15 Centrifuge the extract for 10 minutes at 5000 rpm. Then remove the acetonitrile layer (top) and place in a 10 ml graduated cylinder. Measure the extract volume to the nearest 0.1 ml (should be 3-4 ml). Record this volume on the worksheet in the appropriate area.

7.2.5.16 Pipet 2 ml of the sample from the 10 ml graduated cylinder to an 8 ml glass vial. Add 2 ml of calcium chloride solution (see section 6.4.16) to the vial, shake and let stand for 20 minutes.

7.2.5.17 Remove the supernatant from the calcium chloride treated sample (avoid the flocculated particulates on the bottom) and filter through a 0.2 µm Teflon syringe filter. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.5.18 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.6 Preparation of plant tissue for qualitative/quantitative HPLC analysis

Three separate extractions are performed on each vegetation sample: two with acetonitrile and one with sulfuric acid. The three fractions are injected and analyzed separately. Total concentrations of explosives and degradation products are calculated mathematically.

NOTE: Turn on the freeze dry unit (if it is not already running) and allow the temperature and vacuum to equilibrate to normal running levels during the performance of the following steps.

NOTE: Batches of samples undergoing this preparation shall contain the following QA/QC samples: matrix spike, LCS, and method blank.

7.2.6.1 Retrieve samples and allow to equilibrate to room temperature in the dark and out of air currents.

7.2.6.2 Obtain the appropriate sample worksheet (Attachment 5 - "Preparation of Plant Tissue for Explosives Analysis"). Record on the worksheet, the laboratory number of the samples to be prepared for HPLC analysis, the date, your name, the serial number of the spiking solution to be used, and the concentration of the spiking solution.

Select one sample out of the batch for use in the creation of the matrix spike. Record the number of this sample in the appropriate area in the QC section of the worksheet.

7.2.6.3 Obtain a "Percent Moisture" worksheet (Attachment 6). Record on this worksheet the laboratory number of the samples for this determination, the date, and your name.

7.2.6.4 Using acetonitrile rinsed scissors, or clean gloved hands, cut or tear approximately 30 g of plant tissue into coarse pieces onto clean aluminum foil.

7.2.6.5 Into a plastic boat, weigh out 20 g of plant tissue for the explosives extraction and record this weight on a sample worksheet.. Record this weight on the worksheet in the appropriate place.

7.2.6.6 Set aside enough tissue, for each regular sample, to perform a percent moisture determination as described in section 9.2 of this procedure. The start of this determination must be prompt so that sample moisture is not lost.

7.2.6.7 Place the 20 grams of plant tissue into an acetonitrile rinsed and dried 400 ml glass beaker.

7.2.6.8 Add approximately 200 ml of HPLC water to the beaker. Swirl the plant tissue around for several seconds and then carefully decant the water into a proper waste receptacle. Do not allow any of the plant tissue to be lost.

7.2.6.9 Repeat step 7.2.6.8 twice more, then empty the contents of the beaker onto clean paper towels. Allow the tissue to adequately drain to the point that the tissue can be easily separated from the towels.

7.2.6.10 Place the washed and drained plant tissue in an acetonitrile rinsed ceramic mortar of appropriate size.

7.2.6.11 Add enough liquid nitrogen to the mortar to create a pool in the bottom about ½ inch deep. The nitrogen should be poured over the surface of the tissue to facilitate rapid freezing.

7.2.6.12 Using an acetonitrile rinsed ceramic pestle of appropriate size, gently crush and grind the frozen plant tissue until it reaches the consistency of a loosely flowing powder.

NOTE: Do not allow the plant tissue to warm enough during this step for liquid water to become visible in the mortar. Add more liquid nitrogen if necessary.

7.2.6.13 Using a powder funnel and a stainless steel spatula, quickly transfer the ground tissue from the mortar to an acetonitrile rinsed freeze dry flask (300 ml size).

7.2.6.14 Add 20 ml of HPLC water to the mortar and use to rinse any adhering tissue into the freeze dry flask.

7.2.6.15 Use a HPLC water wash-bottle and rinse all tissue adhering to the mortar, pestle and funnel into the freeze dry flask. Minimize the volume of water used for this step.

7.2.6.16 Use a stainless steel spatula and mix the water and frozen tissue in the freeze dry flask. This is done to keep the tissue mass from freezing into a block.

7.2.6.17 With the tissue in the freeze dry flask, use the 25 mm sawtooth generator probe on the homogenizer to grind the plant tissue to a liquid consistency. Use the speeds and times listed below as a guideline. Keep the generator probe deep enough in the tissue slurry to prevent any material from being ejected from the flask.

1000 rpm---------------2 min.
2500 rpm---------------1.5 min.
5000 rpm---------------1.5 min.
6500 rpm---------------1.5 min.

NOTE: The initial homogenization at 1000 rpm may require additional time in order to breakup the frozen tissue slurry.

7.2.6.18 Sparingly rinse any tissue adhering to the generator probe back into the freeze dry flask with HPLC water.

NOTE: After each plant sample is homogenized, remove the generator probe from the mixer unit, disassemble probe totally and wash the parts thoroughly with detergent, followed by a rinse with distilled water, acetonitrile and then with HPLC water. Then reassemble the generator probe.

7.2.6.19 At this point, if the sample is a Matrix Spike, add 2.0 ml of the appropriate spiking solution (see section 6.4.12) to the flask. The freeze dry flask should then be sealed with Parafilm, the contents swirled for several seconds and then placed in a darkened area for 30 minutes.

7.2.6.20 Place a rubber cap, with its filter and glass adapter in place, on the freeze dry flask.

7.2.6.21 Place the flask containing the tissue slurry in an acetone/dry ice bath and shell freeze the slurry to the walls of the flask. Ensure there are no large masses of frozen tissue in the bottom of the flask and that the slurry is thoroughly frozen.

7.2.6.22 Immediately transport the frozen sample to the freeze dryer, place the sample flask on a free port, carefully apply vacuum to the flask and allow the instrument to equilibrate to its normal operating levels before adding additional samples.

7.2.6.23 Leave sample(s) on the freeze dryer until all tissue is thoroughly dry (probably 24-48 hours for five or more samples). Then carefully remove each sample and seal the top of the glass adapter with Parafilm.

7.2.6.24 Carefully remove the rubber cap from the freeze dry flask. Use a clean spatula and scrape any tissue adhering to the top back into the flask.

7.2.6.25 Use a spatula to push the tissue from the inside walls of the freeze dry flask, to its bottom. Then gently chop and stir the tissue mass until it is finely divided.

7.2.6.26 Carefully transfer the tissue mass (scraping out as much as possible) to a clean glass wide-mouth container. Seal with a Teflon-lined closure and let stand in the dark until ready to proceed.

7.2.6.27 Into a 40 ml wide-mouth glass vial, weigh out 0.5 g of tissue. Record this weight to 0.0001 g the on worksheet.

7.2.6.28 Add 15.0 ml of acetonitrile to the vial and seal with Teflon-lined closure. Record this volume on the worksheet.

7.2.6.29 Suspend the vial in a sonicator bath whose temperature is controlled between 10° C and 25° C such that the water level covers the level of solids/liquids inside the vial. Place cover on the sonicator bath to block out light. Individual vials should not touch each other or the walls of the bath.

7.2.6.30 Sonicate the samples for 18 hours.

7.2.6.31 Remove the sample vials from the sonicator and allow to stand in the dark for 15 minutes.

7.2.6.32 Centrifuge sample vials at 2000 rpm or greater for 30 minutes.

7.2.6.33 Remove as much supernatant as possible from the vial, leaving the tissue pellet undisturbed for further extraction.

7.2.6.34 Place the supernatant in a 250 ml Erlenmeyer flask containing 100 ml of HPLC water. Seal the flask with Parafilm and place flask in dark until ready to proceed with step 7.2.6.37.1.

7.2.6.35 Using the tissue pellet remaining from the previous step, repeat steps 7.2.6.28 through 7.2.6.34. Treat this as a separate fraction. Place the supernatant in a separate Erlenmeyer flask from the first fraction.

7.2.6.36 Place the uncapped vial containing the tissue pellet at a forward leaning angle (facing outward) in the front portion of a functioning darkened hood. Pull the hood sash partially down and allow the pellet to dry out thoroughly. This should be done in a darkened room, away from possible analyte contamination.

7.2.6.36.1 Pipette 10.0 ml of 1+1 sulfuric acid into the vial with and break up the tissue pellet with a stainless steel spatula, being careful to leave all the

tissue in the vial when the spatula is removed. Record this volume on the worksheet.

7.2.6.36.2 Suspend the vial in a sonicator bath whose temperature is regulated at 25° - 30° C and sonicate for 6 hours. The sonicator shall have a cover which blocks out the light.

7.2.6.36.3 Remove the vial from the sonicator bath and centrifuge at 2000 rpm or greater for 30 minutes.

7.2.6.36.4 Remove 5.0 ml of the acidic supernatant and add to 100 ml of 1.07 M sodium phosphate dibasic solution (see section 6.4.14) in an Erlenmeyer flask and swirl. Record the volume of supernatant on the worksheet.

7.2.6.36.5 Seal the flask with Parafilm and place the neutralized supernatant in an explosion-proof refrigerator at 4° C for 12-15 hours.

7.2.6.36.6 Remove the neutralized extract from the refrigerator. If the beaker contains a precipitate or a fluffy suspension, draw off and save most of the free liquid into a clean 250 ml Erlenmeyer flask then proceed to step 7.2.6.36.8.

7.2.6.36.7 If the beaker contains no precipitate or suspension, proceed to step 7.2.6.37.

7.2.6.36.8 Pour the suspension or precipitate layer into a 40 ml glass vial. Rinse the Erlenmeyer flask with HPLC water and add to 40 ml vial. Seal vial with Teflon-lined closure.

7.2.6.36.9 Centrifuge 40 ml vial at 2000 rpm or greater for 30 minutes.

7.2.6.36.10 Remove the supernatant from the precipitate and add to the supernatant removed in step 7.2.6.36.6.

7.2.6.36.11 Place the accumulated supernatant in the dark until ready to proceed with step 7.2.6.37.

7.2.6.37 For each of the three fractions prepared from each sample (two acetonitrile extracts and one sulfuric acid extract) connect in series from top to bottom (using appropriate adapters), one 60 ml reservoir, two Alumina-A SPE (each a 1 g size), and one Porapak-Rdx SPE cartridge (500 mg size). Place this cartridge train onto the vacuum manifold

NOTE: Do not allow the bed of the Porapak-Rdx cartridge to become dry during the conditioning step, between the conditioning and the sample loading steps or during the sample loading step.

7.2.6.38 Condition the cartridges by first pulling 20 ml of acetonitrile (at a flow rate of 2-4 ml/min.) through them, immediately followed by 30 ml of HPLC water at a flow rate of 30 ml/minute. Immediately follow the HPLC water with the sample solution. When the sample flask is empty, rinse it with HPLC water three times and add this to the cartridge reservoir.

NOTE: If the sample being loaded onto the SPE cartridges has as its matrix the 1.07 M sodium phosphate buffer, follow the sample solution with about 70 ml of HPLC water to wash any accumulated salts out of the Porapak-Rdx cartridge.

7.2.6.39 After the sample solution (and wash solution if necessary) has totally passed through the cartridge train, separate the 60 ml reservoir and Alumina-A cartridges from the Porapak-Rdx cartridge which will remain on the vacuum manifold. Dispose of the Alumina-A cartridges properly.

7.2.6.40 Now apply a strong vacuum to the Porapak-Rdx cartridge for about 5 minutes to remove residual water.

7.2.6.41 Remove the Porapak-Rdx cartridge from the vacuum manifold.

7.2.6.42 Position the SPE cartridge over a properly labeled 5 ml volumetric flask (other sizes of volumetric flasks can be used if deemed necessary) with the Luer tip of the cartridge extending into the mouth of the flask.

7.2.6.43 Add 5.0 ml (record on worksheet) of acetonitrile to the SPE cartridge. Apply a lightly pressurized flow of ultrapure nitrogen to the top of the cartridge to start the solvent flowing through the cartridge and into the volumetric flask. Once the solvent flow begins, remove the nitrogen source and allow the solvent flow to be by gravity alone.

7.2.6.44 After the solvent stops dripping from the cartridge, reapply the pressurized nitrogen to the top of the cartridge to force any trapped solvent into the flask.

7.2.6.45 Make the volumetric flask to volume with acetonitrile and mix thoroughly. This extract should be prepared for HPLC analysis on the same day it was

generated, or should be transferred to a glass vial with a Teflon-lined closure and stored in an explosion-proof refrigerator at 4° C until needed.

7.2.6.46 Using precisely measured volumes, place equal amounts of eluant and HPLC grade water (usually 2 ml of each component) in a glass vial, cap with a Teflon-lined closure and mix thoroughly by inversion.

7.2.6.47 Let the mixture stand in the dark at room temperature for 20 minutes.

7.2.6.48 Pass the mixture through a PTFE syringe filter with 0.45 μm pore size. The first 1/3 of the filtrate should be discarded, with the remaining filtrate being apportioned as follows:

If only qualitative or quantitative analysis of the sample is required, then only one autosampler vial containing filtrate is required. If qualitative and quantitative analysis of the sample is required, then two autosampler vials containing filtrate are required.

7.2.6.49 Allow the autosampler vial containing the filtrate to stand in the dark until ready to load onto the autosampler. If filtrates cannot be loaded onto autosampler the same day they are prepared for analysis, they should be stored in an explosion-proof refrigerator at 4° C.

7.2.7 HPLC procedure

After preparation is completed and autosampler vials are filled; load the autosampler, enter the parameters noted below, and start the analysis.

7.2.7.1 Tertiary pump parameters

Pump flow rate: 0.8 ml/min.

Run length: 50.00 minutes

Method end action: Equilibrate at end
Equilibration time: 5.00 minutes

Mobile phase gradient (where phase A is water and phase B is methanol)
Time = 0.00 min.; phase A = 83%; phase B = 17%
Time = 8.00 min.; phase A = 63%; phase B = 37%
Time = 10.00 min.; phase A = 42%; phase B = 58%

Time = 23.00 min.; phase A = 42%; phase B = 58%
Time = 28.00 min.; phase A = 0%; phase B = 100%
Time = 35.00 min.; phase A = 0%; phase B = 100%
Time = 40.00 min.; phase A = 84%; phase B = 16%

(NOTE: Mobile phase percentages and flow rates may be altered prior to an initial calibration to provide the best peak resolution and placement)

7.2.7.2 Autosampler parameters

Sample loop volume: 100 µl

Syringe volume: 1000 µl
Wash cycle volume: 500 µl
Tube volume: 13.0 µl
Viscosity factor: 1
Pre-injection delay: 10 sec.
Post-injection wash: Yes
Automixing volume %: 100%
Automixing type-air mixing: No
Stop output momentary: No
Expel tube volume to vial: No
Pulsed start output: Yes

7.2.7.3 Photodiode array detector parameters

Detector information
Bunch rate: 8 points (2.0 Hz)
Monitor length: 64 bunched points (32.0 seconds)

Polychrom parameters
Screen width: 50.00 minutes
Scan frequency: 16 Hz
Autoprint: Off
Ch A output: Absorbance
Ch A bandwidth: 4 nm
Ch A peak use: Upper-half
Ch A time constant: 0.500 seconds
Ch A offset: 10%

7.3 Calculations and Recording Data

7.3.1 Peak identification is made by commercial chromatography workstation software. Data are stored in individual files for each injection with the extension RP1.

7.3.2 Copy files from a run onto a diskette. Extract and concatenate data into a single file with QBASIC program 16.BAS (or its most recent revision). 16.BAS applies calibration curve factors to peak heights and calculates raw concentration.

7.3.3 Edit the output file from 16.BAS to eliminate mis-identified peaks. Add in peaks which were not identified by the software but were found in manual review of chromatograms.

7.3.4 For vegetation: Enter the weight of the vegetation sample, extraction volumes, and the measured concentration of each target compound for the three fractions into the spreadsheet 'PLANT_TMPLATE.xls' to calculate total concentrations.

Example: A 0.5041 g sample gives readings X1, X2, and X3 on the three fractions for analyte X.

Total X in micgrograms/gram = (X1*5.0 + X2*5.0 +X3*10.0)/0.5041

7.3.5 Interface the edited file with the EBS database. Add weights, volumes, dilution factors, concentration factors, and unit conversion factors to EBS. Review percent recoveries and relative percent differences as calculated by EBS.

7.3.6 Review data and resolve all discrepancies. Print a final copy of the customer report and route it to the supervisor along with the data package for final review.

7.3.7 Store chromatograms, preparation worksheets, EBS printouts, run narratives, notes, logbooks, final reports, and other information as quality assurance records.

8.0 SAFETY

8.1 Care should be taken when handling neat HMX, RDX and TNT since these compounds are classified as explosives. Safety glasses and vinyl gloves should be worn during the use of these compounds. Quantities should be minimized as far as possible.

8.2 Standard laboratory safety precautions should be followed when handling the organic solvents used in this procedure. Safety glasses shall be worn at all times in the laboratory and gloves, appropriate for the solvent being handled, should be worn.

9.0 NOTES

9.1 Method Detection Limit determination in GLP-0018 is done in accordance with Title 40, Code of Federal Regulations, Part 136, Appendix B, "Definition and Procedure for the Determine of the Method Detection Limit" - Revision 1.11.

9.2 Percent moisture by Oven Drying

9.2.1 Obtain the appropriate worksheet (Attachment 5 - "Determination of Percent Moisture by Oven Drying"). Record on the worksheet, laboratory numbers, sample description, and your name.

NOTE: For each sample, the steps (9.2.2-9.2.9) will be identical

9.2.2 Obtain an aluminum weighing boat and label with laboratory number of sample.

9.2.3 Weigh the boat to 0.0001 g and record this as the tare weight (TW) in the appropriate area of the worksheet.

9.2.4 Add the appropriate weight of sample (see list below) to the boat. (Weights may vary depending on the amount of sample material available)

Soil-----------5 g
Sediment----5 g
Gravel-------30 g
Plant---------2 g

9.2.5 Record the weight of the boat plus sample to 0.0001 g and record this as the gross weight (GW) in the appropriate area of the worksheet.

9.2.6 Place the boat containing the sample in an oven at 105° C and leave for 12-15 hours. Record on the worksheet, the date and time the samples were placed in the oven and its temperature at that time.

9.2.7 Remove the boat and allow to equilibrate to room temperature in a dessicator. Record on the worksheet, the date and time the samples were removed from the oven and its temperature at that time.

9.2.8 Remove from the dessicator and weigh the boat and dried sample. Record this weight to 0.0001 g as the dried weight (DW) in the appropriate area of the worksheet.

9.2.9 Calculate the percent moisture of the sample as shown on the worksheet and record the results in the appropriate areas. The formula for the calculation is:

$$\% \text{ Moisture} = \frac{(GW - DW) * 100}{(GW - TW)}$$

9.3 Approximate analyte retention times:

ANALYTE	MINUTES
2,6-Diamino-4-nitrotoluene	12.11
1,3,5-Trinitroso-1,3,5-triazacyclohexane	12.07
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	13.05
2,4-Diamino-6-nitrotoluene	13.53
1-Nitroso-3,5-dinitro-1,3,5-triazacyclohexane	16.11
Hexahydro-1,3,5-trinitro-1,3,5-triazine	15.78
1,3,5-Trinitrobenzene	18.30
1,3-Dinitrobenzene	20.44
3,5-Dinitroaniline	21.82
2,4,6-Trinitrotoluene	23.56
2-Amino-4,6-dinitrotoluene	24.58
4-Amino-2,6-dinitrotoluene	25.43
2,6-Dinitrotoluene	25.85
2,4-Dinitrotoluene	26.44
4,4',6,6'-Tetranitro-2,2'-azoxytoluene	32.69
2,4',6,6'-Tetranitro-2',4-azoxytoluene	32.96
2,2',6,6',Tetranitro-4,4'-azoxytoluene	33.21
2,2'-Dinitro-4,4'-azoxytoluene	34.33

10.0 ATTACHMENTS AND APPENDICES

10.1 Attachment 1
Worksheet - "Preparation of Liquids for Explosives Analysis"

Preparation of Liquids for Explosives Analysis

Start Date:________________ Spike s/n:________________
End Date:________________ Spike concentraton:________________
Analyst:________________ Matrix:___Aqueous___Organic

Laboratory Number	Aliquot (ml)	Dilution Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	AcCN	0.2 um	0.45um

QA/QC Samples

	Sample Vol. (ml)	Spike Vol. (ml)	Final Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	AcCN	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								
Martix Spike Dupl.								

10.2 Attachment 2
Worksheet - "Preconcentration of Liquids by SPE for Explosives Analysis"

Preconcentration of Liquids by SPE for Explosives Analysis

Start Date:____________________ **Spike s/n:**____________________
End Date:____________________ **Spike concentraton:**____________________
Analyst:____________________

Laboratory Number	Sample Vol. (ml)	Eluant Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Spike Vol. (ml)	Final Vol. (ml)	Eluant Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								

10.3 Attachment 3
Worksheet - "Preparation of Solids for Explosives Analysis"

Preparation of Solids for Explosives Analysis

Start Date:____________________
End Date:____________________
Analyst:____________________

Spike s/n:____________________
Spike concentraton:____________________
Matrix: Soil Sediment Gravel

Laboratory Number	Sample Wt. (g)	Extraction Vol. (ml)	Aliquot (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Sample Wt. (g)	Spike Vol. (ml)	Final Vol. (ml)		1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								
Martix Spike Dupl.								

10.4 Attachment 4
Worksheet - "Preparation of Compost Leachates for Explosives Analysis"

Preparation of Compost Leachates for Explosives Analysis

Start Date: ____________________
End Date: ____________________
Analyst: ____________________

Spike s/n: ____________________
Spike concentraton: ____________________

Laboratory Number	Sample Vol. (ml)	Salt Wt. (g)	Extract Vol. (ml)	Dilution Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um

QA/QC Samples

	Sample Vol. (ml)	Salt Wt. (g)	Spike Vol. (ml)	Extract Vol. (ml)	1:1 Mixer Matrix		PTFE Filter	
					Water	$CaCl_2$	0.2 um	0.45um
Method Blank								
LCS								
Matrix Spike								
Martix Spike Dupl.								

10.5 Attachment 5
Worksheet - "Preparation of Plant Tissue for Explosives Analysis"

Preparation of Plants for Explosives Analysis

Start Date:________________
End Date:________________
Analyst:________________

Spike s/n:________________
Spike concentraton:________________
Spike Vol. (ml): MS ______ LCS ______

			Sonication Extraction						Acid Digestion						
Laboratory Number	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant Vol. (ml)	1:1 Mixer Water	1:1 Mixer $CaCl_2$	PTFE Filter 0.2 um	PTFE Filter 0.45um	Acid (ml)	Extract Used (ml)	Eluant (ml)	1:1 Mixer Water	1:1 Mixer $CaCl_2$	PTFE Filter 0.2 um	PTFE Filter 0.45um

QA/QC Samples

			Sonication Extraction						Acid Digestion						
	Wet Wt. (g)	Dry Wt. (g)	AcCN Vol. (ml)	Eluant Vol. (ml)	1:1 Mixer Water	1:1 Mixer $CaCl_2$	PTFE Filter 0.2 um	PTFE Filter 0.45um	Acid (ml)	Extract Used (ml)	Eluant (ml)	1:1 Mixer Water	1:1 Mixer $CaCl_2$	PTFE Filter 0.2 um	PTFE Filter 0.45um
Method Blank															
LCS															
Matrix Spike															

10.6 Attachment 6
Worksheet - "Percent Moisture by Oven Drying"

Percent Moisture by Oven Drying

Analyst:____________________ Sample Type:________________

Initial date / time:________________ Initial oven temp.:____________

Final date / time:________________ Final oven temp.:____________

Laboratory Number	Tare Wt. (TW) Grams	Gross Wt. (GW) Grams	Dried Wt. (DW) Grams	Percent Moisture

Percent Moisture $\frac{(GW - DW) * 100}{(GW - TW)}$

End of Procedure

APPENDIX A-2

Procedures for Metals: Method 200 Series

WP-0031
EPA Method 200.7 for Milan Samples
Metals Analysis by Inductively Coupled Plasma Spectroscopy

1.0 Procedure

1.1 Analyze samples from the Milan project for dissolved metals according to the attached procedure, EPA Method 200.7. The sample collector will have filtered and acidified the metals in the field.

1.2 Examine samples prior to analysis to determine if any precipitate was formed after filtration. If a precipitate is found, coordinate sample preparation and documentation with the metals area team leader.

1.3 The following minor changes to Method 200.7 apply.

1.3.1 Since there was no sample preparation, use no reagent blank.

1.3.2 Read calibration blanks, which are in the same matrix as the samples.

1.3.3 Analyze and report any field blanks submitted by the consignor as routine samples.

1.4 Prepare the interference check sample (IFC) to correspond to the sample matrix for the Milan samples. Determine this sample's composition by running a metals scan on typical samples. Analyze the interference check sample at the beginning of each analytical run.

Note: The only element present that was not part of the routine request list is sulfur. Match this amount in the IFC.

2.0 Recordkeeping

Maintain copies of machine printouts, calibration details, use of standard solutions, details of mixing quality control samples, percent recovery calculations, and any sample preparation worksheets from samples with precipitates.

2/20/98

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Inductively Coupled Plasma—Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes—Method 200.7

1. Scope and Application

1.1 This method may be used for the determination of dissolved, suspended, or total elements in drinking water, surface water, domestic and industrial wastewaters.

1.2 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interference are taken into account. This is especially true when dissolved solids exceed 1500 mg/L. (See 5.)

1.3 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps *must* be taken to correct for potential interference effects. (See 5.)

1.4 Table 1 lists elements for which this method applies along with recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization. Actual working detection limits are sample dependent and as the sample matrix varies, these concentrations may also vary. In time, other elements may be added as more information becomes available and as required.

1.5 Because of the differences between various makes and models of satisfactory instruments, no detailed instrumental operating instructions can be provided. Instead, the analyst is referred to the instructions provided by the manufacturer of the particular instrument.

2. Summary of Method

2.1 The method describes a technique for the simultaneous or sequential multielement determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed and controlled by a computer system. A background correction technique is required to compensate for variable background contribution to the

determination of trace elements. Background must be measured adjacent to analyte lines on samples during analysis. The position selected for the background intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used must be free of spectral interference and reflect the same change in background intensity as occurs at the analyte wavelength measured. Background correction is not required in cases of line broadening where a background correction measurement would actually degrade the analytical result. The possibility of additional interferences named in 5.1 (and tests for their presence as described in 5.2) should also be recognized and appropriate corrections made.

3. Definitions

3.1 Dissolved — Those elements which will pass through a 0.45 μm membrane filter.

3.2 Suspended — Those elements which are retained by a 0.45 μm membrane filter.

3.3 Total — The concentration determined on an unfiltered sample following vigorous digestion (9.3), or the sum of the dissolved plus suspended concentrations. (9.1 plus 9.2.)

3.4 Total recoverable — The concentration determined on an unfiltered sample following treatment with hot, dilute mineral acid (9.4).

3.5 Instrumental detection limit — The concentration equivalent to a signal, due to the analyte, which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength

3.6 Sensitivity — The slope of the analytical curve, i.e. functional relationship between emission intensity and concentration.

3.7 Instrument check standard — A multielement standard of known concentrations prepared by the analyst to monitor and verify instrument performance on a daily basis. (See 7.6.1)

3.8 Interference check sample — A solution containing both interfering and analyte elements of known concentration that can be used to verify background and interelement correction factors. (See 7.6.2)

3.9 Quality control sample — A solution obtained from an outside source having known, concentration values to be used to verify the calibration standards. (See 7.6.3)

3.10 Calibration standards — a series of know standard solutions used by the analyst for calibration of the instrument (i.e., preparation of the analytical curve). (See 7.4)

3.11 Linear dynamic range — The concentration range over which the analytical curve remains linear.

3.12 Reagent blank — A volume of deionized, distilled water containing the same acid matrix as the calibration standards carried through the entire analytical scheme. (See 7.5.2)

3.13 Calibration blank — A volume of deionized, distilled water acidified with HNO_3 and HCl. (See 7.5.1)

3.14 Method of standard addition — The standard addition technique involves the use of the unknown and the unknown plus a known amount of standard. (See 10.6.1)

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified (14.7, 14.8 and 14.9) for the information of the analyst.

5. Interferences

5.1 Several types of interference effects may contribute to inaccuracies in the determination of trace elements. They can be summarized as follows:

5.1.1 Spectral interferences can be categorized as 1) overlap of a spectral line from another element; 2) unresolved overlap of molecular band spectra; 3) background contribution from continuous or recombination phenomena; and 4) background contribution from stray light from the line emission of high concentration elements. The first of these effects can be compensated by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated by a background correction adjacent to the analyte line. In addition, users of simultaneous multielement instrumentation must assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample but for which there is no channel in the instrument array. Listed in Table 2 are some interference effects for the recommended wavelengths given in Table 1. The data in Table 2 are intended for use only as a rudimentary guide for the indication of potential spectral interferences. For this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed.

The interference information, which was collected at the Ames Laboratory,[1] is expressed at analyte concentration eqivalents (i.e. false analyte concentrations) arising from 100 mg/L of the interferent element. The suggested use of this information is as follows: Assume that arsenic (at 193.696 nm) is to be determined in a sample containing approximately 10 mg/L of aluminum. According to Table 2, 100 mg/L of aluminum would yield a false signal for arsenic equivalent to approximately 1.3 mg/L. Therefore, 10 mg/L of aluminum would result in a false signal for arsenic equivalent to approximately 0.13 mg/L. The reader is cautioned that other analytical systems may exhibit somewhat different levels of interference than those shown in Table 2, and that the interference effects must be evaluated for each individual system.

Only those interferents listed were investigated and the blank spaces in Table 2 indicate that measurable interferences were not observed for the interferent concentrations listed in Table 3. Generally, interferences were discernible if they produced peaks or background shifts corresponding to 2-5% of the peaks generated by the

[1]Ames Laboratory, USDOE, Iowa State University, Ames Iowa 50011

analyte concentrations also listed in Table 3.

At present, information on the listed silver and potassium wavelengths are not available but it has been reported that second order energy from the magnesium 383.231 nm wavelength interferes with the listed potassium line at 766.491 nm.

5.1.2 *Physical interferences* are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples which may contain high dissolved solids and/or acid concentrations. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they must be reduced by dilution of the sample and/or utilization of standard addition techniques. Another problem which can occur from high dissolved solids is salt buildup at the tip of the nebulizer. This affects aersol flow-rate causing instrumental drift. Wetting the argon prior to nebulization, the use of a tip washer, or sample dilution have been used to control this problem. Also, it has been reported that better control of the argon flow rate improves instrument performance. This is accomplished by the use of mass flow controllers.

5.1.3 *Chemical Interferences* are characterized by molecular compound formation, ionization effects and solute vaporization effects. Normally these effects are not pronounced with the ICP technique, however, if observed they can be minimized by careful selection of operating conditions (that is, incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte element.

5.2 It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests, as outlined in 5.2.1 through 5.2.4, will ensure the analyst that neither positive nor negative interference effects are operative on any of the analyte elements thereby distorting the accuracy of the reported values.

5.2.1 *Serial dilution*—If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrumental detection limit after dilution), an analysis of a dilution should agree within 5 % of the original determination (or within some acceptable control limit (14.3) that has been established for that matrix). If not, a chemical or physical interference effect should be suspected.

5.2.2 *Spike addition*—The recovery of a spike addition added at a minimum level of 10X the instrumental detection limit (maximum 100X) to the original determination should be recovered to within 90 to 110 percent or within the established control limit for that matrix. If not, a matrix effect should be suspected. The use of a standard addition analysis procedure can usually compensate for this effect. *Caution:* The standard addition technique does not detect coincident spectral overlap. If suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended. (See 5.2.3)

5.2.3 *Comparison with alternate method of analysis*—When investigating a new sample matrix, comparison tests may be performed with other analytical techniques such as atomic absorption spectrometry, or other approved methodology.

5.2.4 *Wavelength scanning of analyte line region*—If the appropriate equipment is available, wavelength scanning can be performed to detect potential spectral interferences.

6. Apparatus

6.1 Inductively Coupled Plasma-Atomic Emission Spectrometer.

6.1.1 Computer controlled atomic emission spectrometer with background correction.

6.1.2 Radiofrequency generator.

6.1.3 Argon gas supply, welding grade or better.

6.2 Operating conditions — Because of the differences between various makes and models of satisfactory instruments, no detailed operating instructions can be provided. Instead, the analyst should follow the instructions provided by the manufacturer of the particular instrument. Sensitivity, instrumental detection limit, precision, linear dynamic range, and interference effects must be investigated and established for each individual analyte line on that particular instrument. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions used satisfy the analytical requirements and to maintain quality control data confirming instrument performance and analytical results.

7. Reagents and standards

7.1 Acids used in the preparation of standards and for sample processing must be ultra-high purity grade or equivalent. Redistilled acids are acceptable.

7.1.1 Acetic acid, conc. (sp gr 1.06).

7.1.2 Hydrochloric acid, conc. (sp gr 1.19).

7.1.3 Hydrochloric acid, (1+1): Add 500 mL conc. HCl (sp gr 1.19) to 400 mL deionized, distilled water and dilute to 1 liter.

7.1.4 Nitric acid, conc. (sp gr 1.41).

7.1.5 Nitric acid,(1+1): Add 500 mL conc. HNO_3 (sp. gr 1.41) to 400 mL deionized, distilled water and dilute to 1 liter.

7.2 *Dionized, distilled water:* Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized, distilled water for the preparation of all reagents, calibration standards and as dilution water. The purity of this water must be equivalent to ASTM Type II reagent water of Specification D 1193 (14.6).

7.3 *Standard stock solutions* may be purchased or prepared from ultra high purity grade chemicals or metals. All salts must be dried for 1 h at 105°C unless otherwise specified. (CAUTION: Many metal salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling.) Typical stock solution preparation procedures follow:

7.3.1 Aluminum solution, stock, 1 mL = 100 µg Al: Dissolve 0.100 g of aluminum metal in an acid mixture of 4 mL of (1+1) HCl and 1 mL of conc. HNO_3 in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a liter flask, add an additional 10 mL of (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.2 Antimony solution stock, 1 mL = 100 µg Sb: Dissolve 0.2669 g $K(SbO)C_4H_4O_6$ in deionized distilled water, add 10 mL (1+1) HCl and dilute to 1000 mL with deionized, distilled water.

7.3.3 Arsenic solution, stock, 1 mL = 100 μg As: Dissolve 0.1320 g of As_2O_3 in 100 mL of deionized, distilled water containing 0.4 g NaOH. Acidify the solution with 2 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.4 Barium solution, stock, 1 mL = 100 μg Ba: Dissolve 0.1516 g $BaCl_2$ (dried at 250°C for 2 hrs) in 10 mL deionized, distilled water with 1 mL (1+1) HCl. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.5 Beryllium solution, stock, 1 mL = 100 μg Be: *Do not dry.* Dissolve 1.966 g $BeSO_4 \cdot 4$ $4H_2O$, in deionized, distilled water, add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.6 Boron solution, stock, 1 mL = 100 μg B: *Do not dry.* Dissolve 0.5716 g anhydrous H_3BO_3 in deionized distilled water dilute to 1,000 mL. Use a reagent meeting ACS specifications, keep the bottle tightly stoppered and store in a desiccator to prevent the entrance of atmospheric moisture.

7.3.7 Cadmium solution, stock, 1 mL = 100 μg Cd: Dissolve 0.1142 g CdO in a minimum amount of (1+1) HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.8 Calcium solution, stock, 1 mL = 100 μg Ca: Suspend 0.2498 g $CaCO_3$ dried at 180°C for 1 h before weighing in deionized, distilled water and dissolve cautiously with a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.9 Chromium solution, stock, 1 mL = 100 μg Cr: Dissolve 0.1923 g of CrO_3 in deionized, distilled water. When solution is complete, acidify with 10 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.10 Cobalt solution, stock, 1 mL = 100 μg Co: Dissolve 0.1000 g of cobalt metal in a minimum amount of (1+1) HNO_3. Add 10.0 mL (1+1) HCl and dilute to 1,000 mL with deionized, distilled water.

7.3.11 Copper solution, stock, 1 mL = 100 μg Cu: Dissolve 0.1252 g CuO in a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.12 Iron solution, stock, 1 mL = 100 μg Fe: Dissolve 0.1430 g Fe_2O_3 in a warm mixture of 20 mL (1+1) HCl and 2 mL of conc. HNO_3. Cool, add an additional 5 mL of conc. HNO_3 and dilute to 1000 mL with deionized, distilled water.

7.3.13 Lead solution, stock, 1 mL = 100 μg Pb: Dissolve 0.1599 g $Pb(NO_3)_2$ in minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.14 Magnesium solution, stock, 1 mL = 100 μg Mg: Dissolve 0.1658 g MgO in a minimum amount of (1+1) HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.15 Manganese solution, stock, 1 mL = 100 μg Mn: Dissolve 0.1000 g of manganese metal in the acid mixture 10 mL conc. HCl and 1 mL conc. HNO_3, and dilute to 1,000 mL with deionized, distilled water.

7.3.16 Molybdenum solution, stock, 1 mL = 100 μg Mo: Dissolve 0.2043 g $(NH_4)_2MoO_4$ in deionized, distilled water and dilute to 1,000 mL.

7.3.17 Nickel solution, stock, 1 mL = 100 μg Ni: Dissolve 0.1000 g of nickel metal in 10 mL hot conc. HNO_3, cool and dilute to 1,000 mL with deionized, distilled water.

7.3.18 Potassium solution, stock, 1 mL = 100 μg K: Dissolve 0.1907 g KCl, dried at 110°C, in deionized, distilled water dilute to 1,000 mL.

7.3.19 Selenium solution, stock, 1 mL = 100 μg Se: *Do not dry.* Dissolve 0.1727 g H_2SeO_3 (actual assay 94.6%) in deionized, distilled water and dilute to 1,000 mL.

7.3.20 Silica solution, stock, 1 mL = 100 μg SiO_2: *Do not dry.* Dissolve 0.4730 g $Na_2SiO_3 \cdot 9H_2O$ in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.21 Silver solution, stock, 1 mL = 100 μg Ag: Dissolve 0.1575 g $AgNO_3$ in 100 mL of deionized, distilled water and 10 mL conc. HNO_3. Dilute to 1,000 mL with deionized, distilled water.

7.3.22 Sodium solution, stock, 1 mL = 100 μg Na: Dissolve 0.2542 g NaCl in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.23 Thallium solution, stock, 1 mL = 100 μg Tl: Dissolve 0.1303 g $TlNO_3$ in deionized, distilled water. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.24 Vanadium solution, stock, 1 mL = 100 μg V: Dissolve 0.2297 NH_4VO_3 in a minimum amount of conc. HNO_3. Heat to increase rate of dissolution. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.3.25 Zinc solution, stock, 1 mL = 100 μg Zn: Dissolve 0.1245 g ZnO in a minimum amount of dilute HNO_3. Add 10.0 mL conc. HNO_3 and dilute to 1,000 mL with deionized, distilled water.

7.4 *Mixed calibration standard solutions*—Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks. (See 7.4.1 thru 7.4.5) Add 2 mL of (1+1) HCl and dilute to 100 mL with deionized, distilled water. (See Notes 1 and 6.) Prior to preparing the mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards that the elements are compatible and stable. Transfer the mixed standard solutions to a FEP fluorocarbon or unused polyethylene bottle for storage. Fresh mixed standards should be prepared as needed with the realization that concentration can change on aging. Calibration standards must be initially verified using a quality control sample and monitored weekly for stability (See 7.6.3). Although not specifically required, some typical calibration standard combinations follow when using those specific wavelengths listed in Table 1.

7.4.1 Mixed standard solution I—Manganese, beryllium, cadmium, lead, and zinc.

7.4.2 Mixed standard solution II—Barium, copper, iron, vanadium, and cobalt.

7.4.3 Mixed standard solution III—Molybdenum, silica, arsenic, and selenium.

7.4.4 Mixed standard solution IV—Calcium, sodium, potassium, aluminum, chromium and nickel

7.4.5 Mixed standard solution V—Antimony, boron, magnesium, silver, and thallium.

NOTE 1: If the addition of silver to the recommended acid combination results in an initial precipitation, add 15 mL of deionized distilled water and warm the flask until the solution clears. Cool and dilute to 100 mL with deionized, distilled water. For this acid combination the silver concentration should be limited to 2 mg/L. Silver under these conditions is stable in a tap water matrix for 30 days. Higher concentrations of silver require additional HCl.

7.5 Two types of blanks are required for the analysis. The calibration blank (3.13) is used in establishing the analytical curve while the reagent blank (3.12) is used to correct for possible contamination resulting from varying amounts of the acids used in the sample processing.

7.5.1 The calibration blank is prepared by diluting 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to 100 mL with deionized, distilled water. (See Note 6.) Prepare a sufficient quantity to be used to flush the system between standards and samples.

7.5.2 The reagent blank must con-contain all the reagents and in the same volumes as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

7.6 In addition to the calibration standards, an instrument check standard (3.7), an interference check sample (3.8) and a quality control sample (3.9) are also required for the analyses.

7.6.1 The *instrument check standard* is prepared by the analyst by combining compatible elements at a concentration equivalent to the midpoint of their respective calibration curves. (See 12.1.1)

7.6.2 The *interference check sample* is prepared by the analyst in the following manner. Select a representative sample which contains minimal concentrations of the analytes of interest by known concentration of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at the approximate concentration of either 100 $\mu g/L$ or 5 times the estimated detection limits given in Table 1. (For effluent samples of expected high concentrations, spike at an appropriate level.) If the type of samples analyzed are varied, a synthetically prepared sample may be used if the above criteria and intent are met. A limited supply of a synthetic interference check sample will be available from the Quality Assurance Branch of EMSL-Cincinnati. (See 12.1.2)

7.6.3 The *quality control sample* should be prepared in the same acid matrix as the calibration standards at a concentration near 1 mg/L and in accordance with the instructions provided by the supplier. The Quality Assurance Branch of EMSL-Cincinnati will either supply a quality control sample or information where one of equal quality can be procured. (See 12.1.3)

8. Sample handling an preservation

8.1 For the determination of trace elements, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus which the sample contacts are all sources of potential contamination. Sample containers can introduce either positive or negative errors in the measurement of trace elements by (a) contributing contaminants through leaching or surface desorption and (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. Laboratory glassware including the sample bottle (whether polyethylene, polyproplyene or FEP-fluorocarbon) should be thoroughly washed with detergent and tap water; rinsed with (1+1) nitric acid, tap water, (1+1) hydrochloric acid, tap and finally deionized, distilled water in that order (See Notes 2 and 3).

NOTE 2: Chromic acid may be useful to remove organic deposits from glassware; however, the analyst should be be cautioned that the glassware must be thoroughly rinsed with water to remove the last traces of chromium. This is especially important if chromium is to be included in the analytical scheme. A commercial product, NOCHROMIX, available from Godax Laboratories, 6 Varick St., New York, NY 10013, may be used in place of chromic acid. Chomic acid should not be used with plastic bottles.

NOTE 3: If it can be documented through an active analytical quality control program using spiked samples and reagent blanks, that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure.

8.2 Before collection of the sample a decision must be made as to the type of data desired, that is dissolved, suspended or total, so that the appropriate preservation and pretreatment steps may be accomplished. Filtration, acid preservation, etc., are to be performed at the time the sample is collected or as soon as possible thereafter.

8.2.1 For the determination of dissolved elements the sample must be filtered through a 0.45-μm membrane filter as soon as practical after collection. (Glass or plastic filtering apparatus are recommended to avoid possible contamination.) Use the first 50-100 mL to rinse the filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO_3 to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample.

8.2.2 For the determination of suspended elements a measured volume of unpreserved sample must be filtered through a 0.45-μm membrane filter as soon as practical after collection. The filter plus suspended material should be transferred to a suitable container for storage and/or shipment. No preservative is required.

8.2.3 For the determination of total or total recoverable elements, the sample is acidified with (1+1) HNO_3 to pH 2 or less as soon as possible, preferable at the time of collection. The sample is not filtered before processing.

9. Sample Preparation

9.1 For the determinations of dissolved elements, the filtered, preserved sample may often be analyzed as received. The acid matrix and concentration of the samples and calibration standards must be the same. (See Note 6.) If a precipitate formed upon acidification of the sample or during transit or storage, it must be redissolved before the analysis by adding additional acid and/or by heat as described in 9.3.

9.2 For the determination of suspended elements, transfer the membrane filter containing the insoluble material to a 150-mL Griffin beaker and add 4 mL conc. HNO_3. Cover the

beaker with a watch glass and heat gently. The wam acid will soon dissolve the membrane.

Increase the temperature of the hot plate and digest the material. When the acid has nearly evaporated, cool the beaker and watch glass and add another 3 mL of conc. HNO_3. Cover and continue heating until the digestion is complete, generally indicated by a light colored digestate. Evaporate to near dryness (2 mL), cool, add 10 mL HCl (1+1) and 15 mL deionized, distilled water per 100 mL dilution and warm the beaker gently for 15 min. to dissolve any precipitated or residue material. Allow to cool, wash down the watch glass and beaker walls with deionized distilled water and filter the sample to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the volume based on the expected concentrations of elements present. This volume will vary depending on the elements to be determined (See Note 6). The sample is now ready for analysis. Concentrations so determined shall be reported as "suspended."

NOTE 4: In place of filtering, the sample after diluting and mixing may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.

9.3 For the determination of total elements, choose a measured, volume of the well mixed acid preserved sample appropriate for the expected level of elements and transfer to a Griffin beaker. (See Note 5.) Add 3 mL of conc. HNO_3. Place the beaker on a hot plate and evaporate to near dryness cautiously, making certain that the sample does not boil and that no area of the bottom of the beaker is allowed to go dry. Cool the beaker and add another 5 mL portion of conc. HNO_3. Cover the beaker with a watch glass and return to the hot plate. Increase the temperature of the hot plate so that a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing.) Again, evaporate to near dryness and cool the beaker. Add 10 mL of 1+1 HCl and 15 mL of deionized, distilled water per 100 mL of final solution and warm the beaker gently for 15 min. to dissolve any precipitate or residue resulting from evaporation. Allow to cool, wash down the beaker walls and watch glass with deionized distilled water and filter the sample to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the sample to a predetermined volume based on the expected concentrations of elements present. The sample is now ready for analysis (See Note 6). Concentrations so determined shall be reported as "total."

NOTE 5: If low determinations of boron are critical, quartz glassware should be use.

NOTE 6: If the sample analysis solution has a different acid concentration from that given in 9.4, but does not introduce a physical interference or affect the analytical result, the same calibration standards may be used.

9.4 For the determination of total recoverable elements, choose a measured volume of a well mixed, acid preserved sample appropriate for the expected level of elements and transfer to a Griffin beaker. (See Note 5.) Add 2 mL of (1+1) HNO_3 and 10 mL of (1+1) HCl to the sample and heat on a steam bath or hot plate until the volume has been reduced to near 25 mL making certain the sample does not boil. After this treatment, cool the sample and filter to remove insoluble material that could clog the nebulizer. (See Note 4.) Adjust the volume to 100 mL and mix. The sample is now ready for analysis. Concentrations so determined shall be reported as "total."

10. Procedure

10.1 Set up instrument with proper operating parameters established in 6.2. The instrument must be allowed to become thermally stable before beginning. This usually requires at least 30 min. of operation prior to calibration.

10.2 Initiate appropriate operating configuration of computer.

10.3 Profile and calibrate instrument according to instrument manufacturer's recommended procedures, using the typical mixed calibration standard solutions described in 7.4. Flush the system with the calibration blank (7.5.1) between each standard. (See Note 7.) (The use of the average intensity of multiple exposures for both standardization and sample analysis has been found to reduce random error.)

NOTE 7: For boron concentrations greater than 500 $\mu g/L$ extended flush times of 1 to 2 min. may be required.

10.4 Before beginning the sample run, reanalyze the highest mixed calibration standard as if it were a sample. Concentration values obtained should not deviate from the actual values by more than $\pm$ 5 percent (or the established control limits whichever is lower). If they do, follow the recommendations of the instrument manufacturer to correct for this condition.

10.5 Begin the sample run flushing the system with the calibration blank solution (7.5.1) between each sample. (See Note 7.) Analyze the instrument check standard (7.6.1) and the calibration blank (7.5.1) each 10 samples.

10.6 If it has been found that method of standard addition are required, the following procedure is recommended.

10.6.1 The standard addition technique (14.2) involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal thus producing a different slope from that of the calibration standards. It will not correct for additive interference which causes a baseline shift. The simplest version of this technique is the single-addition method. The procedure is as follows. Two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a small volume V_s of a standard analyte solution of concentration c_s. To the second (labeled B) is added the same volume V_s of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration c_x is calculated:

$$c_x = \frac{S_B V_S c_S}{(S_A - S_B)\, V_x}$$

where S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_S and c_S should be chosen so that S_A is roughly twice S_B on the average. It is best if V_S is made much less than V_x, and thus c_S is much greater than c_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear
2. The chemical form of the analyte added must respond the same as the analyte in the sample.

3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

11. Calculation

11.1 Reagent blanks (7.5.2) should be subtracted from all samples. This is particularly important for digested samples requiring large quantities of acids to complete the digestion.

11.2 If dilutions were performed, the appropriate factor must be applied to sample values.

11.3 Data should be rounded to the thousandth place and all results should be reported in mg/L up to three significant figures.

12. Quality Control (Instrumental)

12.1 Check the instrument standardization by analyzing appropriate quality control check standards as follow:

12.1.1 Analyze an appropriate instrument check standard (7.6.1) containing the elements of interest at a frequency of 10%. This check standard is used to determine instrument drift. If agreement is not within ±5% of the expected values or within the established control limits, whichever is lower, the analysis is out of control. The analysis should be terminated, the problem corrected, and the instrument recalibrated.

Analyze the calibration blank (7.5.1) at a frequency of 10%. The result should be within the established control limits of two standard deviations of the mean value. If not, repeat the analysis two more times and average the three results. If the average is not within the control limit, terminate the analysis, correct the problem and recalibrate the instrument.

12.1.2 To verify interelement and background correction factors analyze the interference check sample (7.6.2) at the beginning, end, and at periodic intervals throughout the sample run. Results should fall within the established control limits of 1.5 times the standard deviation of the mean value. If not, terminate the analysis, correct the problem and recalibrate the instrument.

12.1.3 A quality control sample (7.6.3) obtained from an outside source must first be used for the initial verification of the calibration standards. A fresh dilution of this sample shall be anlayzed every week thereafter to monitor their stability. If the results are not within ±5% of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new stock standard and a new calibration standard and repeat the calibration.

Precision and Accuracy

13.1 In an EPA round robin phase 1 study, seven laboratories applied the ICP technique to acid-distilled water matrices that had been dosed with various metal concentrates. Table 4 lists the true value, the mean reported value and the mean % relative standard deviation.

References

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7. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No 77-206, Aug. 1977

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Table 1. Recommended Wavelengths [1] and Estimated Instrumental Detection Limits

Element	Wavelength, nm	Estimated detection limit, µg/L[2]
Aluminum	308.215	45
Arsenic	193.696	53
Antimony	206.833	32
Barium	455.403	2
Beryllium	313.042	0.3
Boron	249.773	5
Cadmium	226.502	4
Calcium	317.933	10
Chromium	267.716	7
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Lead	220.353	42
Magnesium	279.079	30
Manganese	257.610	2
Molybdenum	202.030	8
Nickel	231.604	15
Potassium	766.491	see[3]
Selenium	196.026	75
Silica (SiO_2)	288.158	58
Silver	328.068	7
Sodium	588.995	29
Thallium	190.864	40
Vanadium	292.402	8
Zinc	213.856	2

[1] The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference. (See 5.1.1.).

[2] The estimated instrumental detection limits as shown are taken from "Inductively Coupled Plasma-Atomic Emission Spectroscopy-Prominent Lines," EPA-600/4-79-017. They are given as a guide for an instrumental limit. The actual method detection limits are sample dependent and may vary as the sample matrix varies.

[3] Highly dependent on operating conditions and plasma position.

Table 2. Analyte Concentration Equivalents (mg/L) Arising From Interferents at the 100 mg/L Level

Analyte	Wavelength, nm	Interferent									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	—	—	—	—	—	—	0.21	—	—	1.4
Antimony	206.833	0.47	—	2.9	—	0.08	—	—	—	.25	0.45
Arsenic	193.696	1.3	—	0.44	—	—	—	—	—	—	1.1
Barium	455.403	—	—	—	—	—	—	—	—	—	—
Beryllium	313.042	—	—	—	—	—	—	—	—	0.04	0.05
Boron	249.773	0.04	—	—	—	0.32	—	—	—	—	—
Cadmium	226.502	—	—	—	—	0.03	—	—	0.02	—	—
Calcium	317.933	—	—	0.08	—	0.01	0.01	0.04	—	0.03	0.03
Chromium	267.716	—	—	—	—	0.003	—	0.04	—	—	0.04
Cobalt	228.616	—	—	0.03	—	0.005	—	—	0.03	0.15	—
Copper	324.754	—	—	—	—	0.003	—	—	—	0.05	0.02
Iron	259.940	—	—	—	—	—	—	0.12	—	—	—
Lead	220.353	0.17	—	—	—	—	—	—	—	—	—
Magnesium	279.079	—	0.02	0.11	—	0.13	—	0.25	—	0.07	0.12
Manganese	257.610	0.005	—	0.01	—	0.002	0.002	—	—	—	—
Molybdenum	202.030	0.05	—	—	—	0.03	—	—	—	—	—
Nickel	231.604	—	—	—	—	—	—	—	—	—	—
Selenium	196.026	0.23	—	—	—	0.09	—	—	—	—	—
Silicon	288.158	—	—	0.07	—	—	—	—	—	—	0.01
Sodium	588.995	—	—	—	—	—	—	—	—	0.08	—
Thallium	190.864	0.30	—	—	—	—	—	—	—	—	—
Vanadium	292.402	—	—	0.05	—	0.005	—	—	—	0.02	—
Zinc	213.856	—	—	—	0.14	—	—	—	0.29	—	—

Table 3. Interferent and Analyte Elemental Concentrations Used for Interference Measurements in Table 2.

Analytes	(mg/L)	Interferents	(mg/L)
Al	10	Al	1000
As	10	Ca	1000
B	10	Cr	200
Ba	1	Cu	200
Be	1	Fe	1000
Ca	1	Mg	1000
Cd	10	Mn	200
Co	1	Ni	200
Cr	1	Ti	200
Cu	1	V	200
Fe	1		
Mg	1		
Mn	1		
Mo	10		
Na	10		
Ni	10		
Pb	10		
Sb	10		
Se	10		
Si	1		
Tl	10		
V	1		
Zn	10		

Table 4. ICP Precision and Accuracy Data

	Sample # 1			Sample #2			Sample #3		
Element	True Value µg/L	Mean Reported Value µg/L	Mean Percent RSD	True Value µg/L	Mean Reported Value µg/L	Mean Percent RSD	True Value µg/L	Mean Reported Value µg/L	Mean Percent RSD
Be	750	733	6.2	20	20	9.8	180	176	5.2
Mn	350	345	2.7	15	15	6.7	100	99	3.3
V	750	749	1.8	70	69	2.9	170	169	1.1
As	200	208	7.5	22	19	23	60	63	17
Cr	150	149	3.8	10	10	18	50	50	3.3
Cu	250	235	5.1	11	11	40	70	67	7.9
Fe	600	594	3.0	20	19	15	180	178	6.0
Al	700	696	5.6	60	62	33	160	161	13
Cd	50	48	12	2.5	2.9	16	14	13	16
Co	500	512	10	20	20	4.1	120	108	21
Ni	250	245	5.8	30	28	11	60	55	14
Pb	250	236	16	24	30	32	80	80	14
Zn	200	201	5.6	16	19	45	80	82	9.4
Se	40	32	21.9	6	8.5	42	10	8.5	8.3

Not all elements were analyzed by all laboratories.

APPENDIX A-3

Procedure for YSI 600 Sonde

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1.3	Sensor Specifications	1-3
1.4	How to Use This Manual	1-5
2.0	Initial Setup	2-1
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3.2	Calibration Procedures	3-1

1. INTRODUCTION

1.1 DESCRIPTION

The 600XL Environmental Monitoring System is a multiparameter, water quality measurement, and data collection system. It is intended for use in research, assessment, and regulatory compliance applications.

Measurement parameters include:

- Dissolved Oxygen
- Conductivity
- Specific Conductance
- Salinity
- Total Dissolved Solids
- Resistivity
- Temperature
- pH
- ORP
- Depth
- Level

The YSI Model 600XL is similar in appearance and performance to the original YSI Model 600 series, but is characterized by three significant enhancements. First, the Model 600XL offers field replaceable sensors. Second, the instrument can be configured with a factory-installed depth sensor module. Third, the unit is now available with an ORP sensor. Like the original Model 600, the 600XL has significant similarities to the YSI Model 6000 series, but also differs from that larger instrument in several ways. First, the Model 600XL does not have internal battery capability, and therefore must be powered from an external power source such as an AC adapter, battery pack, or terminal device. Second, the Model 600XL has no internal logging capability, and therefore the Model 600XL must be used with a terminal, data logger, data collection platform, or computer. Finally, several sensors, such as turbidity, nitrate, and ammonium, which are available on the Model 6000, cannot be used with the 600XL.

The Model 600XL is ideal for profiling and monitoring water conditions in industrial and waste water effluents, lakes, rivers, wetlands, estuaries, coastal waters, and monitoring wells. It can be left unattended for weeks at a time with measurement parameters sampled at your setup interval and data transmitted to your computer or logging device. The Model 600XL can be used 200 feet below the water's surface or in as little as a few inches of water. The fast sensor response of the Model 600XL makes it ideal for vertical profiling. Its very small size allows it to fit down 2 inch diameter monitoring wells.

The Model 600XL is equipped with YSI's patented Rapid Pulse Dissolved Oxygen Sensor which exhibits low stirring dependence, and therefore provides accurate results without an expensive and bulky stirrer. Because stirring is not required, external battery life is extended. In addition, because of the nature of the technology, sensor drift caused by passive fouling is minimized.

The Model 600XL communicates with an ASCII terminal or a computer with a terminal emulation program. Use of the 600XL with our 610 D and 610 DM display/loggers provides an ideal system for profiling or spot sampling.

Every Model 600XL comes with IBM-compatible PC based software for simple and convenient setup and data handling. Reports and plots are automatically generated and their presentation easily customized. Data is easily exported to any spreadsheet program for more sophisticated data processing.

The RS-232C and SDI-12 interfaces provide maximum versatility for system networking and real time data collection. Several Model 600XL units are easily installed as a network, providing valuable water quality data at a variety of locations. For real time results, the Model 600XL can interface to radio telemetry systems, satellite, modem and cellular phone data collection platforms.

The Model 600XL is available with an economical built-in cable of various lengths, or with a sonde-mounted connector. Optional interface cables in several lengths are available for interfacing with a computer or terminal. These cables are waterproof at the sonde end and can be used in the lab or field.

See Appendix D for a complete list of accessories and calibration reagents.

1.2 GENERAL SPECIFICATIONS

See also Section 1.3 Sensor Specifications.

Operating Environment
Medium: fresh, sea, or polluted water
Temperature: -5 to +45 °C
Depth: 0 to 200 (61 meters)

Storage Temperature: -40 to +60 °C

Material: PVC, Stainless Steel

Dimensions and weight with a 50 foot integral cable.
Diameter: 1.6 inches (4.06 cm)
Length: 14 inches (35.56 cm)
Weight: 4.9 pounds (2.22 kg)

Dimensions with depth sensor bulkhead installed and no attached cable.
Diameter: 1.6 inches (4.06 cm)
Length: 20.75 inches (52.7 cm)
Weight: 1.75 pounds (0.8 kg)

Computer Interface
RS-232C
SDI-12

Software
IBM PC compatible computer, 3 1/2 or 5 1/4 inch, high or low density floppy disk drive.
Minimum RAM requirement: 256 K bytes
Optional graphic adapter for plotting

Power
External 12 VDC (8 to 13.8 VDC)

1.3 SENSOR SPECIFICATIONS

The following are typical performance specifications for each sensor.

Depth - Medium
Sensor Type...........Stainless steel strain gauge
Range......................0 to 200 ft (61 m)
Accuracy................+/- 0.4 ft (0.12 m)
Resolution..............0.001 ft (0.001 m)

Depth - Shallow
Sensor Type..............Stainless steel strain gauge
Range.........................0-30 ft (9.1 m)
Accuracy .... +/- 0.06 ft (0.018 m)
Range.........................0.001 ft (0.001 m)

Temperature
Sensor Type...........Thermistor
Range......................-5 to 45 °C
Accuracy................+/- 0.15 °C (optional configuration at +/- 0.05 °C)
Resolution..............0.01 °C

Dissolved Oxygen, % saturation
Sensor Type...........Rapid Pulse - Clark type, polarographic
Range......................0 to 200 % air saturation
Accuracy................+/- 2 % air saturation
Resolution..............0.1 % air saturation

Dissolved Oxygen, mg/L (Calculated from % air saturation, temperature and salinity)
Sensor Type........... Rapid Pulse - Clark type polarographic
Range.....................0 to 20 mg/L
Accuracy................+/- 0.2 mg/L
Resolution..............0.01 mg/L

Conductivity*
Sensor Type...........4 electrode cell
Range.....................0 to 100 mS/cm
Accuracy................+/- 0.5% of reading + 0.001 mS/cm
Resolution..............0.01 mS/cm or 1 uS/cm

Salinity
Sensor Type............Calculated from conductivity and temperature
Range......................0 to 70 ppt
Accuracy.................+/- 1.0% of reading or 0.1 ppt, whichever is greater
Resolution...............0.01 ppt

pH
Sensor Type...........Glass combination electrode
Range.....................2 to 14 units
Accuracy................+/- 0.2 units
Resolution..............0.01 units

pH - Low Ionic Strength
Sensor Type...........Glass combination electrode with open junction and low impedance glass
Range.....................2 to 14 units
Accuracy................+/- 0.2 units
Resolution..............0.01 units

ORP
Sensor type............ Platinum ring
Range......................-999 to 999 mv
Accuracy................+/-20 mv
Resolution...............0.1 mv

---

* Report outputs of specific conductance (conductivity corrected to 25 C), resistivity, and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (Ed 1989).

1.4 HOW TO USE THIS MANUAL

This manual provides information for operating and maintaining the Model 600XL Environmental Monitoring System. Sections 1 through 3 provide an overview of setup, calibration, and operational procedures. These first three chapters should provide enough information for you to understand the basic capabilities of the 600XL system and begin sampling. Sections 4 through 9 provide a more detailed explanation of system operations, software, principles of operation, maintenance, and performance troubleshooting. Appendices A-G provide information about health and safety, warranty, accessories, and options.

NOTE: Because of the many features and applications of this versatile product, some sections of this manual may not apply to the specific system you have purchased.

This manual is organized to let you quickly understand and operate the 600XL system. However, it cannot be stressed too strongly that informed and safe operation is more than just knowing which buttons to push. An understanding of the principles of operation, calibration techniques, and system setup is necessary to obtain accurate and meaningful results.

Regular maintenance is required to keep the 600XL functioning properly. Precautions regarding the handling of reagents are also essential for the safety of system operators (see Appendix A for health and safety information).

The early parts of this manual will teach you how to get the 600XL system running. Additional topics are included to help you understand the science it employs, how to use it most effectively and safely, and how to keep it operating correctly.

The 600XL can be purchased with external battery or power supply capability. Additionally, all probes, cables and accessories can be ordered as options or ordered together as a system.

If you have any questions about this product or its application, please contact our customer service department or authorized dealer for assistance. See Appendix C for contact information.

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2. INITIAL SETUP

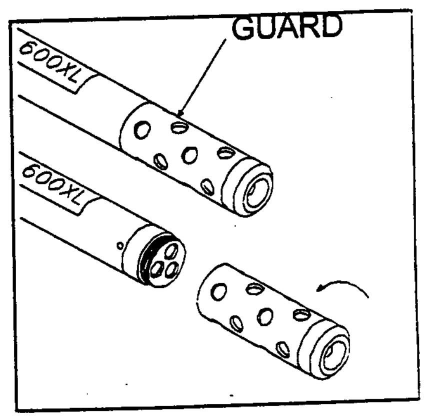
2.1 UNPACKING

Remove the instrument from the shipping container. Be careful not to discard any parts or supplies. Check off all items on the packing list and inspect all assemblies and components for damage. If any parts are damaged or missing, contact your representative immediately. If you do not know from which dealer your 600XL was purchased, refer to Appendix C for contact information.

NOTE: Reagents for the 600XL are not packaged in the same carton as the instrument. These materials must be ordered separately and will arrive in a separate package.

2.2 SONDE SETUP

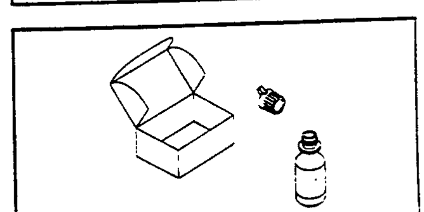
SENSORS



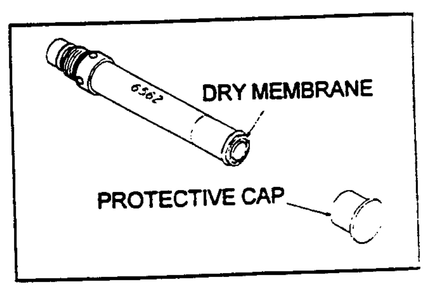
1. Remove the Model 600XL probe guard by hand.

2. NOTE: Step 2 is for the preparation of the 6562 dissolved oxygen probe only. To install other probes, proceed to step 3.

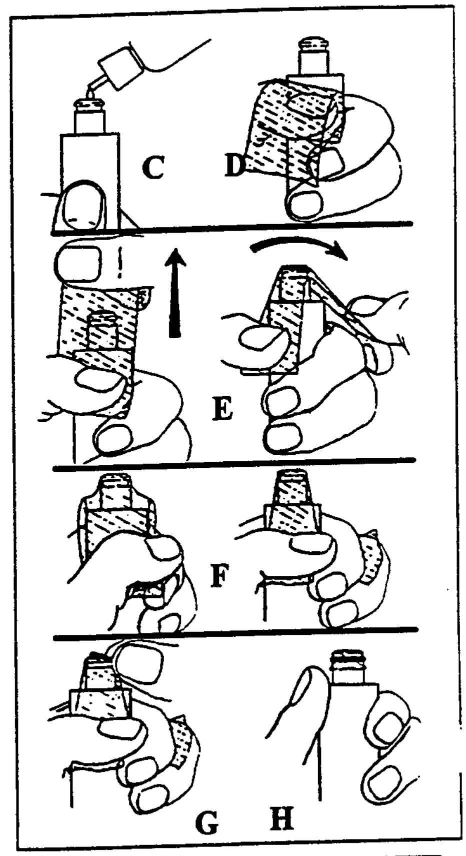
   A. Open the membrane kit and prepare electrolyte. Dissolve the KCl in the dropper bottle by filling it to the neck with distilled water and shaking until the solid is fully dissolved. After dissolution is complete, wait 10-15 minutes unit the solution is free of bubbles.



   B. Remove protective cap and the dry membrane from the 600XL dissolved oxygen probe. NOTE: The dissolved oxygen probe is shipped with a protective dry membrane on the sensor tip. It is very important not to scratch or contaminate the sensor tip. Handle the new probe with care. Avoid touching or hitting of the sensor tip.



C. Hold the probe in a vertical position and apply a few drops of KCl solution to the tip. The fluid should completely fill the small moat around the electrodes and form a meniscus on the tip of the sensor. Be sure no air bubbles are stuck to the face of the sensor. If necessary, shake off the electrolyte and start over.



D. Secure a membrane between your left thumb and the probe body. Always handle the membrane with care, touching it at the ends only.

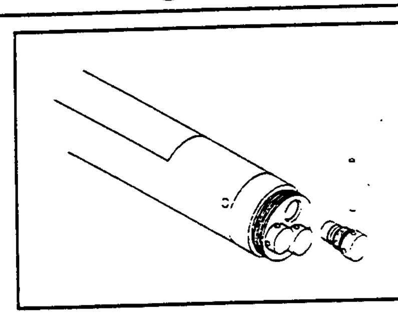
E. With the thumb and forefinger of your right hand, grasp the free end of the membrane. With one continuous motion, gently stretch it up, over, and down the other side of the sensor. The membrane should conform to the face of the sensor.

F. Secure the end of the membrane under the forefinger of your left hand.

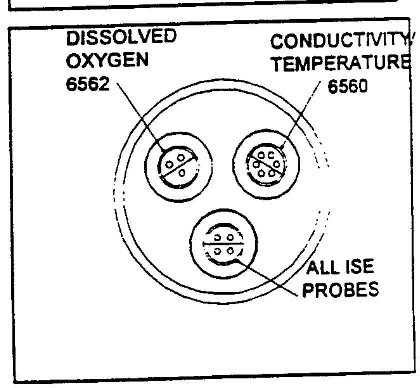
G. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface with your fingers. There should be no wrinkles or trapped air bubbles. Small wrinkles may be removed by lightly tugging on the edges of the membrane. If bubbles are present, remove the membrane and repeat steps C-G.

H. Trim off any excess membrane with a sharp knife or scissors. Make sure the temperature sensor is not covered by excess membrane. Being careful not to get water in the connector, rinse off the excess KCl solution.

NOTE: Some users find it more convenient to mount the sensor vertically in a vise with rubber jaws while applying the electrolyte and membrane.

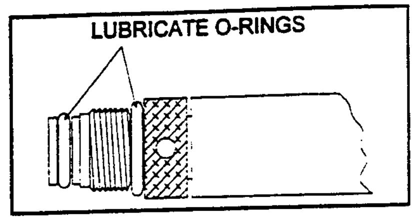


3. Using the probe installation tool supplied in the 6570 maintenance kit, remove the port plugs and locate the port with the connector corresponding to the probe you wish to install.



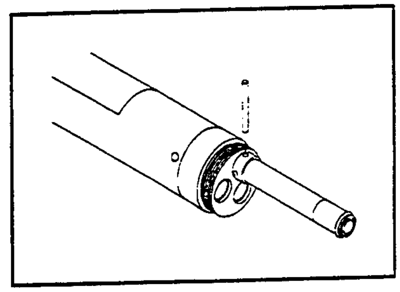
Note: 6562 Dissolved oxygen probe = 3-pin connector
6560 Conductivity/Temperature = 6-pin connector
6561 pH probe = 4 pin connector
6563 ORP probe = 4 pin connector
6565 Combination pH/ORP probe = 4 pin connector
6564 LIS pH probe = 4 pin connector
6567 combination LIS pH/ORP probe = 4 pin connector

4. Apply a thin coat of O-ring lubricant (supplied in the YSI 6570 maintenance kit) to the O-rings on the connector side of the probe.

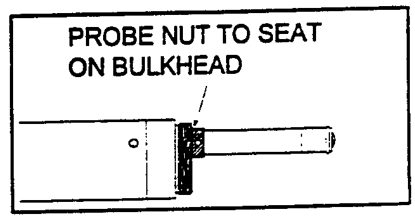


5. NOTE: Before installing probe into sonde, be sure probe port is free of moisture.

Insert the probe into the correct port and gently rotate the probe until the two connectors align.

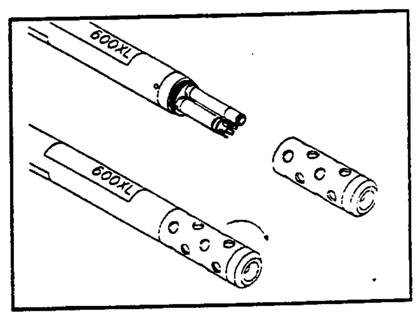


6. With connectors aligned, screw down the probe nut using the probe installation tool. **CAUTION**: Use care not to cross thread the probe nut. Seat nut on face of bulkhead. Do not over tighten.



7..Repeat steps 3-6 for all remaining probes.

8. Replace the 600XL probe guard.



CABLES

Some versions of the Model 600XL have permanently attached cables. If your 600XL has a cable which is non-detachable (no stainless steel connector), parts of this section will not be relevant.

To attach a cable to the 600XL, remove the waterproof cap from the sonde connector and set it aside carefully for later reassembly. Now connect your YSI PC interface cable to the sonde connector. A built-in "key" will ensure proper pin alignment; rotate the cable gently until the "key" engages and then tighten the connectors together by rotating clockwise.

The other end of the cable is a military-style 8-pin connector. This connector plugs directly into the 610 D and 610 DM display/loggers. Most other applications will require the use of an adapter. For example, to connect the 600XL to a computer, use a YSI 6095 MS8 to DB-9 adapter.

POWER

Some type of external power supply is required to power the 600XL sonde. For laboratory setup and calibration with the sonde interfaced to a computer, the YSI 6038 (110 VAC) or 6037 (220 VAC) is ideal. Most adapters include a short pigtail for power that plugs into the power supply. After attaching the three pin connector on the power supply to the pigtail, simply plug the power supply into the appropriate outlet. If you have purchased a 610-series display/logger for use with your 600XL, attachment of the cable to the 610 will allow your sonde to be powered from the batteries in the display/logger or from the 610 power supply if its batteries are not fully charged.

3. BASIC OPERATION

In the previous Section, you learned how to install probes and set up the PC6000 and 600XL sonde software. In this Section, you will learn how to calibrate and run the Model 600XL and how to view your data on a computer display. If you choose to use your 600XL with a 610-series display/logger, refer to the operations manual for the 610 to obtain similar instructions to those provided below.

3.1 CALIBRATION TIPS

WARNING: Reagents used to calibrate and check this instrument may be hazardous to your health. Refer to Appendix A for health and safety information.

Before you begin the calibration procedures outlined below, you may find it helpful to follow some or all of these calibration tips.

1. Remove the sonde stainless steel weight on the bottom of the sonde guard by turning it counterclockwise. This allows the calibration solutions access to the probes with minimal displacement of fluid within the calibration cup. Additionally, carry-over from one solution to the next is reduced.

2. Fill a large bucket with ambient temperature water for rinsing the sonde between calibration solutions.

3. Have several clean, absorbent paper towels or cotton cloths available to dry the sonde between rinses and calibration solutions. It is important to remove as much residual liquid as possible from the sonde after each rinse. Shake the sonde to remove excess rinse water from the inside of the guard. Then dry the outside of the sonde and guard. Drying the sonde and probes in this way reduces carry-over contamination of calibrator solutions and increases the accuracy of the calibration, particularly lower conductivity calibration standards.

4. It is not necessary to remove the probe guard to rinse and dry the probes between calibration solutions. The inaccuracy resulting from simply rinsing the probe compartment and drying the outside of the sonde is minimal.

3.2 CALIBRATION PROCEDURES

WARNING: Calibration reagents may be hazardous to your health. Refer to Appendix A for health and safety information.

A calibration cup is supplied with the Model 600XL. Because the calibration cup fits over the outside of the sonde sensor guard, it is not recommended or necessary to remove the guard to calibrate the sensors. Follow the procedures below to calibrate the sensors. Only *basic* DO percent

saturation, Conductivity, pH, and Depth calibration procedures are discussed in this section. Temperature does not require calibration and is, therefore, not included in the Calibrate menu. ORP calibration is required only infrequently and is discussed in Section 4.2. For more detailed calibration procedures, which can be used to enhance the accuracy of some measurements, see Section 4.2.

From the sonde Main menu select **2. Calibrate.** The Calibrate menu will be displayed.

```
------------------Calibrate------------------------
1. Conductivity          4. ISE1-pH
2. Dissolved Oxy         5. ISE2-Orp
3. Pressure-Abs

Select option (0 for previous menu):
```

Selection of any of the parameters from the Calibrate menu listing will require the user to input a numerical value and then press Enter. For example, for calibration of specific conductance, the following display will be shown during the calibration sequence.

```
Enter SpCond in mS/cm (10):
```

The number in parentheses is the default value of this parameter and will be used in the calibration if *only* Enter is pressed without typing in another value. Similar prompts will be displayed calibration of all parameters, but for some sensors, such as pH, no default values are provided. In these cases, the user must input a numerical value and then press Enter.

After the calibration value is input and Enteris pressed, a real-time display similar to the following will then appear on the screen. Note that all parameters which have been enabled will appear - not just the one being calibrated at the moment. The user should carefully observe the stabilization of the readings of the parameter which is being calibrated and, when the readings are stable for approximately 30 seconds, press Enter to implement the calibration.

```
==========================================================
 Temp   SpCond   Cond      Sal
  C     mS/cm    mS/cm     ppt
----------------------------------------------------------
To calibrate, press <Enter> when the readings are stable.
 23.00   10.0     10.0      15.7

Select option (0 for previous menu)
```

NOTE: If an ERROR message appears, begin the calibration procedure again. Be certain that the value you enter for the calibration standard is correct. Also see Section 8, Troubleshooting for more information on error messages.

CAUTION: Be certain to **immerse the entire sonde** in solution standards for calibration of all parameters. Most calibrations require readings not only from the sensor being calibrated but also from the temperature sensor.

Specific start-up calibration procedures for all sensors which commonly require calibration are provided in the following paragraphs of this section. Remember that these are basic protocols designed to get the user up and running with regard to the 600XL. The more-detailed discussion of sensor calibration found in Section 4.2 should be examined prior to use of the instrument in the field.

NOTE: If the particular sensor listed is not installed in your sonde, proceed to the next sensor until the calibration protocol is complete.

CONDUCTIVITY

NOTE: This procedure calibrates not only conductivity, but also specific conductance, salinity, and total dissolved solids.

Place approximately 300 mL of conductivity standard in a clean and dry calibration cup. The conductivity standard you choose should be within the same conductivity range as the water you are preparing to sample. However, we do not recommend using standard less than 1 mS/cm. For example:

- For fresh water choose a 1 mS/cm conductivity standard.
- For brackish water choose a 10 mS/cm conductivity standard.
- For sea water choose a 50 mS/cm conductivity standard.

Caution: Before proceeding insure that the sensor is as dry as possible. Ideally, rinse the conductivity sensor with a small amount of standard that can be discarded. Be certain that you avoid cross contamination of standard solutions with other solutions. Make certain that there are no salt deposits around the oxygen and pH/ORP probes, particularly if you are employing standards of low conductivity.

Without removing the sonde guard, *carefully* immerse the probe end of the sonde into the solution. Gently rotate and/or move the sonde up and down to remove any bubbles from the conductivity cell. The probe must be completely immersed past its vent hole.

Allow at least one minute for temperature equilibration before proceeding.

From the Calibrate menu, select **1. Conductivity** to access the Conductivity calibration procedure and then **1. SpCond.** to access the specific conductance calibration procedure. Enter the calibration value of the standard you are using (mS/cm at 25 C) and press Enter. The current values of all enabled sensors will appear on the screen and will change with time as they stabilize.

Observe the readings under Specific Conductance or Conductivity and when they show no significant change for approximately 30 seconds, press Enter. The screen will indicate that the calibration has been accepted and prompt you to press Enter again to return to the Calibrate menu.

Rinse the sonde in tap or purified water and dry the sonde.

pH 2-POINT

Place approximately 200 mL of pH 7 buffer in a clean calibration cup. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

From the **Calibrate** menu, select **4. ISE1 pH** to access the pH calibration choices and then **2. 2-Point**. Press Enter and input the value of the buffer (7 in this case) at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. The display will indicate that the calibration is accepted.

After the pH 7 calibration is complete, press Enter again, as instructed on the screen, to continue.

Rinse the sonde in water and dry the sonde before proceeding to next step.

Place approximately 200 mL of a second pH buffer solution in a clean calibration cup. The second buffer might be pH 4 if the sample is expected to be acidic or pH 10 if the sample is expected to be basic. *Carefully* immerse the probe end of the sonde into the solution.

Allow at least 1 minute for temperature equilibration before proceeding.

Press Enter and input the value of the second buffer at the prompt. Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize in the solution. Observe the readings under pH and when they show no significant change for approximately 30 seconds, press Enter. After the second value calibration is complete, press Enter again, as instructed on the screen, to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

Thoroughly rinse and dry the calibration cups for future use.

DISSOLVED OXYGEN

Place approximately 1/8 inch of water or a wet sponge in the bottom of the calibration cup. Place the probe end of the sonde into the calibration cup. Make certain that the DO and the temperature probes are not immersed in the water. Wait approximately 10 minutes for the air in the calibration cup to become water saturated and for the temperatures of the thermistor and the oxygen probe to equilibrate. Make certain that the calibration cup is vented to the atmosphere.

From the **Calibrate** menu, select **2. Dissolved Oxy** to access the DO % calibration procedure.

Enter the current barometric pressure in mm of Hg . *Remember that barometer readings which appear in meteorological reports are generally corrected to sea level and are not useful for you calibration procedure unless they are uncorrected.*

NOTE: Inches of Hg x 25.4 mm/inch = mm Hg

Press Enter and the current values of all enabled sensors will appear on the screen and will change with time as they stabilize. Observe the readings under DO % and when they show no significant change for approximately 30 seconds, press Enter. The screen will indicate that the calibration has been accepted and prompt you to press Enter again to return to the Calibrate menu.

Rinse the sonde in water and dry the sonde.

NOTE: Calibration of dissolved oxygen in the DO % procedure also results in calibration of the DO mg/L mode and vice versa.

NOTE: The above procedure is designed to calibrate your dissolved oxygen sensor for use in sampling applications where the sensor is being pulsed continuously in the Run mode because both "Auto sleep" and "Wait for DO" functions have been disabled as described in Section 2. If your 600XL is to be used in a monitoring application in which data is being captured to a computer or data collection platform, "Auto sleep" and "Wait for DO" will be activated and the calibration displays will be somewhat different. See Section 4 for details.

DEPTH

Following the DO calibration, leave the sonde in water-saturated air. Make certain that the sonde is not submerged in water for the depth calibration.

From the Calibrate menu, select **4. Depth** to access the depth calibration procedure. Input 0.00 or some known sensor offset in feet. Press Enter and monitor the stabilization of the depth readings with time. When no significant change occurs for approximately 30 seconds, press Enter to confirm the calibration and zero the sensor with regard to current barometric pressure.

After depth is zeroed, press Enter again, as instructed on the screen, to return to the Calibrate menu.

The sensors are now calibrated. Press Esc until the sonde Main menu is displayed.

APPENDIX A-4

Procedure for Temperature: Method 170.1

Temperature - Method 170.1

1.0 Procedure

Perform temperature measurements in accordance with "Temperature" Method 170.1 (Thermometric) as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

3.0 Quality Control Samples

None

TEMPERATURE

Method 170.1 (Thermometric)

STORET NO. 00010

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
2. Summary of Method
   2.1 Temperature measurements may be made with any good grade of mercury-filled or dial type centigrade thermometer, or a thermistor.
3. Comments
   3.1 Measurement device should be routinely checked against a precision thermometer certified by the National Bureau of Standards.
4. Precision and Accuracy
   4.1 Precision and accuracy for this method have not been determined.
5. Reference
   5.1 The procedure to be used for this determination is found in:
   Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 125, Method 212 (1975).

Approved for NPDES
Issued 1971

APPENDIX A-5

Procedure for pH: Method 150.1

pH - Method 150.1 or Method 150.2

1.0 Procedure

Perform pH measurements by either Method 150.1 or 150.2 (attached) as appropriate.

2.0 Recordkeeping

Retain all machine printouts, worksheets, and notes.

3.0 Quality Control Samples

Periodically, reanalyze calibration buffers.

pH

Method 150.1 (Electrometric)

STORET NO.
Determined on site 00400
Laboratory 00403

1. Scope and Application
   1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes and acid rain (atmospheric deposition).
2. Summary of Method
   2.1 The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode.
3. Sample Handling and Preservation
   3.1 Samples should be analyzed as soon as possible preferably in the field at the time of sampling.
   3.2 High-purity waters and waters not at equilibrium with the atmosphere are subject to changes when exposed to the atmosphere, therefore the sample containers should be filled completely and kept sealed prior to analysis.
4. Interferences
   4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity.
   4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.
   4.3 Coatings of oily material or particulate matter can impair electrode response. These coatings can usually be removed by gentle wiping or detergent washing, followed by distilled water rinsing. An additional treatment with hydrochloric acid (1 + 9) may be necessary to remove any remaining film.
   4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.
5. Apparatus
   5.1 pH Meter-laboratory or field model. A wide variety of instruments are commercially available with various specifications and optional equipment.

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5.2 Glass electrode.

5.3 Reference electrode–a calomel, silver-silver chloride or other reference electrode of constant potential may be used.

NOTE 1: Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel type filling materials that require minimal maintenance.

5.4 Magnetic stirrer and Teflon-coated stirring bar.

5.5 Thermometer or temperature sensor for automatic compensation.

6. Reagents

6.1 Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is necessary.

6.1.1 Preparation of reference solutions from these salts require some special precautions and handling[1] such as low conductivity dilution water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.

6.2 Secondary standard buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, that have been validated by comparison to NBS standards, are recommended for routine use.

7. Calibration

7.1 Because of the wide variety of pH meters and accessories, detailed operating procedures cannot be incorporated into this method. Each analyst must be acquainted with the operation of each system and familiar with all instrument functions. Special attention to care of the electrodes is recommended.

7.2 Each instrument/electrode system must be calibrated at a minimum of two points that bracket the expected pH of the samples and are approximately three pH units or more apart.

7.2.1 Various instrument designs may involve use of a "balance" or "standardize" dial and/or a slope adjustment as outlined in the manufacturer's instructions. Repeat adjustments on successive portions of the two buffer solutions as outlined in procedure 8.2 until readings are within 0.05 pH units of the buffer solution value.

8. Procedure

8.1 Standardize the meter and electrode system as outlined in Section 7.

8.2 Place the sample or buffer solution in a clean glass beaker using a sufficient volume to cover the sensing elements of the electrodes and to give adequate clearance for the magnetic stirring bar.

8.2.1 If field measurements are being made the electrodes may be immersed directly in the sample stream to an adequate depth and moved in a manner to insure sufficient sample movement across the electrode sensing element as indicated by drift free (<0.1 pH) readings.

8.3 If the sample temperature differs by more than 2°C from the buffer solution the measured pH values must be corrected. Instruments are equipped with automatic or manual

[1]National Bureau of Standards Special Publication 260.

compensators that electronically adjust for temperature differences. Refer to manufacturer's instructions.

8.4 After rinsing and gently wiping the electrodes, if necessary, immerse them into the sample beaker or sample stream and stir at a constant rate to provide homogeneity and suspension of solids. Rate of stirring should minimize the air transfer rate at the air water interface of the sample. Note and record sample pH and temperature. Repeat measurement on successive volumes of sample until values differ by less than 0.1 pH units. Two or three volume changes are usually sufficient.

8.5 For acid rain samples it is most important that the magnetic stirrer is not used. Instead, swirl the sample gently for a few seconds after the introduction of the electrode(s). Allow the electrode(s) to equilibrate. The air-water interface should not be disturbed while measurement is being made. If the sample is not in equilibrium with the atmosphere, pH values will change as the dissolved gases are either absorbed or desorbed. Record sample pH and temperature.

9. Calculation

9.1 pH meters read directly in pH units. Report pH to the nearest 0.1 unit and temperature to the nearest °C.

10. Precision and Accuracy

10.1 Forty-four analysts in twenty laboratories analyzed six synthetic water samples containing exact increments of hydrogen-hydroxyl ions, with the following results:

pH Units	Standard Deviation pH Units	Accuracy as Bias, %	Accuracy as Bias, pH Units
3.5	0.10	−0.29	−0.01
3.5	0.11	−0.00	
7.1	0.20	+1.01	+0.07
7.2	0.18	−0.03	−0.002
8.0	0.13	−0.12	−0.01
8.0	0.12	+0.16	+0.01

(FWPCA Method Study 1. Mineral and Physical Analyses)

10.2 In a single laboratory (EMSL), using surface water samples at an average pH of 7.7, the standard deviation was ±0.1.

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United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

pH, Continuous Monitoring (Electrometric)—Method 150.2

1. Scope and Application

1.1 This method is applicable to the continuous pH measurement of drinking, surface, and saline waters, domestic and industrial waste waters.

2. Summary of Method

2.1 The pH of a sample is determined electrometrically using a glass electrode with a reference electrode or a single combination electrode.

3. Sample Handling and Preservation

3.1 The composition of the water or waste contacting the measuring electrode system must be representative of the total flow from the water body.

4. Interferences

4.1 The glass electrode, in general, is not subject to solution interferences from color, turbidity, colloidal matter, oxidants, reductants or high salinity

4.2 Sodium error at pH levels greater than 10 can be reduced or eliminated by using a "low sodium error" electrode.

4.3 Manually inspect the conditions of the electrodes every 30 days for coating by oily materials or buildup of lime. If oil and grease and/or scale buildup are not present, this time interval may be extended.

4.3.1 Coatings of oil, grease and very fine solids can impair electrode response. These can usually be removed by gentle wiping and detergent washing. The use of flow-through electrode housings which provide higher flow velocity helps to prevent the coating action.

4.3.2 Heavy particulate matter such as lime accumulation can be removed by careful scrubbing or immersion in dilute (1+9) hydrochloric acid. Continuous monitoring under these conditions benefits from ultrasonic or other in-line continuous cleaning methods.

4.4 Temperature effects on the electrometric measurement of pH arise from two sources. The first is caused by the change in electrode output at various temperatures. This interference can be controlled with instruments having temperature compensation or by calibrating the electrode-instrument system at the temperature of the samples. For best results, meters having automatic temperature compensation should be calibrated with solutions within 5°C of the temperature of the stream to be measured. The second source is the change of pH inherent in the sample at various temperatures. This error is sample dependent and cannot be controlled, it should therefore be noted by reporting both the pH and temperature at the time of analysis.

5. Apparatus

5.1 pH Monitor - A wide variety of instruments are commercially available with various specifications and optional equipment. For unattended use, the monitor should be equipped with automatic or fixed

temperature compensation and with a recorder or alarm function.

5.2 Glass electrode - with shielded cable between electrode and monitor unless preamplification is used.

5.3 Reference electrode - a reference electrode with a constant potential and with either a visible electrolyte or viscous gel fill.

NOTE 1: Combination electrodes incorporating both measuring and reference functions are convenient to use and are available with solid, gel-type filling materials that require minimal maintenance.

5.4 Temperature sensor - for automatic compensator covering general ambient temperature range.

5.5 Electrode mounting - to hold electrodes; may be flow through (for small flows), pipe mounted or immersion.

6. Reagents

6.1 Primary standard buffer salts are available from the National Bureau of Standards and should be used in situations where extreme accuracy is required.

6.1.1 Preparation of reference solutions from these salts require some special precautions and handling[1] such as low conductivity dilution water, drying ovens, and carbon dioxide free purge gas. These solutions should be replaced at least once each month.

6.2 Secondary buffers may be prepared from NBS salts or purchased as a solution from commercial vendors. Use of these commercially available solutions, which have been validated by comparison to NBS standards, is recommended for routine operation. These buffers may be retained for at least six months if kept stoppered.

7. Calibration

7.1 Immersion type electrodes - easily removed from mounting.

7.1.1 The electrode should be calibrated at a minimum of two points that bracket the expected pH of the water/waste and are approximately three pH units or more apart.

7.1.2 Repeat calibration adjustments on successive portions of the two buffer solutions until readings are within ±0.05 pH units of the buffer value. If calibration problems occur, see 4.3.

7.1.3 Because of the wide variety of instruments available, no detailed operating instructions are provided. Instead, the analyst should refer to the particular manufacturer's instructions.

7.1.4 Calibration against two buffers should be carried out at least daily. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data supports a longer period between calibration.

7.2 Immersion type electrodes - not easily removed from mounting.

7.2.1 Collect a grab sample of the flowing material from a point as close to the electrode as possible. Measure the pH of this grab sample as quickly as possible with a laboratory - type pH meter. Adjust the calibration control of the continuous monitor to the reading obtained.

7.2.2 The temperature and condition of the grab sample must remain constant until its pH has been measured by the laboratory pH meter. The temperature of the sample should be measured and the temperature compensator of the laboratory pH meter adjusted.

7.2.3 The laboratory - type pH meter should be calibrated prior to use against two buffers as outlined in 7.1.

7.2.4 The continuous pH monitoring system should be initially calibrated against two buffers as outlined in 7.1 before being placed into service. Recalibration (every 30 days) at two points is recommended if at all possible to ensure the measuring electrode is in working order. If this is not possible, the use of electrode testing features for a broken or malfunctioning electrode should be considered when purchasing the equipment.

7.2.5 The indirect calibration should be carried out at least once a day. If the pH of the fluid being measured fluctuates considerably, the calibration should be carried out more often. Calibration frequencies may be relaxed if historical data support a longer period between calibration.

7.2.6 If the electrode can be removed from the system, but with difficulty, it should be directly calibrated as in 7.1 at least once a month.

7.3 Flow-through type electrode - easily removed from its mounting.

7.3.1 Calibrate using buffers as in 7.1. The buffers to be used may be the process stream itself as one buffer, and as a second buffer after adjustment of pH by addition of an acid or base. This will provide the larger volumes necessary to calibrate this type electrode.

7.3.2 Since the velocity of sample flow-through a flow through electrode can produce an offset error in pH reading, the user must have data on hand to show that the offset is known and compensation has been accomplished.

7.4 Flow-through type electrode - not easily removed from its mounting.

7.4.1 Calibrate as in 7.2.

7.4.2 Quality control data must be on hand to show the user is aware of possible sample flow velocity effects.

8. Procedure

8.1 Calibrate the monitor and electrode system as outlined in Section 7.

8.2 Follow the manufacturer's recommendation for operation and installation of the system.

8.3 In wastewaters, the electrode may require periodic cleaning. After manual cleaning, the electrode should be calibrated as in 7.1 or 7.2 before returning to service.

8.4 The electrode must be placed so that the water or waste flowing past the electrode is representative of the system.

9. Calculations

9.1 pH meters read directly in pH units. Reports pH to the nearest 0.1 unit and temperature to the nearest °C.

10. Precision and Accuracy

10.1 Because of the wide variability of equipment and conditions and the changeable character of the pH of many process waters and wastes, the precision of this method is probably less than that of Method 150.1; however, a precision of 0.1 pH unit

[1] National Bureau of Standards Special Publication

should be attainable in the range of pH 6.0 to 8.0. Accuracy data for continuous monitoring equipment are not available at this time.

Bibliography

1. Annual Book of ASTM Standards, Part 31, "Water" Standard 1293-78, Method D, p. 226 (1981).

APPENDIX A-6

Procedure for Oxidation Reduction Potential: Method 2580

2580 OXIDATION-REDUCTION POTENTIAL (ORP) (PROPOSED)*

2580 A. Introduction

1. Significance

Oxidation and reduction (redox) reactions mediate the behavior of many chemical constituents in drinking, process, and wastewaters as well as most aquatic compartments of the environment.[1-5] The reactivities and mobilities of important elements in biological systems (e.g., Fe, S, N, and C), as well as those of a number of other metallic elements, depend strongly on redox conditions. Reactions involving both electrons and protons are pH- and Eh-dependent; therefore, chemical reactions in aqueous media often can be characterized by pH and Eh together with the activity of dissolved chemical species. Like pH, Eh represents an intensity factor. It does not characterize the capacity (i.e., poise) of the system for oxidation or reduction.

The potential difference measured in a solution between an inert indicator electrode and the standard hydrogen electrode should not be equated to Eh, a thermodynamic property, of the solution. The assumption of a reversible chemical equilibrium, fast electrode kinetics, and the lack of interfering reactions at the electrode surface are essential for such an interpretation. These conditions rarely, if ever, are met in natural water.

Thus, although measurement of Eh in water is relatively straightforward, many factors limit the interpretation of these values. These factors include irreversible reactions, electrode poisoning, the presence of multiple redox couples, very small exchange currents, and inert redox couples. Eh values measured in the field correlate poorly with Eh values calculated from the redox couples present. Nevertheless, measurement of redox potential, when properly performed and interpreted, is useful in developing a more complete understanding of water chemistry.

* Approved by Standard Methods Committee, 1990.

2. Sampling and Storage

Do not store samples; analyze on collection. Minimize both atmospheric contact and delay in analysis.

3. References

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2580 B. Oxidation-Reduction Potential Measurement in Clean Water

1. General Discussion

a. Principle: Electrometric measurements are made by potentiometric determination of electron activity (or intensity) with an inert indicator electrode and a suitable reference electrode. Ideally, the indicator electrode will serve as either an electron donor or acceptor with respect to electroactive oxidized or reduced chemical species in solution. At redox equilibrium, the potential difference between the ideal indicator electrode and the reference electrode equals the redox potential of the system. However, inert indicator electrodes that behave ideally in all aqueous systems, particularly in natural waters, do not exist. Electrodes made of platinum are most commonly used for Eh measurements. They have limitations,[1] as do alternative materials such as gold and graphite.

The standard hydrogen reference electrode is fragile and impractical for routine laboratory and field use. Therefore, silver:silver-chloride or calomel reference electrodes are used commonly. The redox potential measurement is corrected for the difference between the potential of the reference electrode and that of the standard hydrogen electrode. See Section 4500-H^+, pH Value.

It is not possible to calibrate Eh electrodes over a range of redox potentials (as is done with pH electrodes). Instead, standard solutions that exhibit both chemical stability and known redox potentials for specific indicator electrodes are used to check electrode response at the temperature of measurement.

The potential of the platinum (Pt) Eh electrode versus the Ag/AgCl reference electrode with KCl electrolyte in ZoBell's solution ($3 \times 10^{-3}M$ potassium ferrocyanide and $3 \times 10^{-3}M$ potassium ferricyanide in $0.1M$ KCl)[2] has been measured as a function of temperature.[3] Good agreement was obtained between Eh values measured with this electrode pair in ZoBell's solution and those calculated from the stability constants at 8 to 85°C. The potential of the ZoBell's solution with this electrode configuration as a function of temperature can be calculated:[4]

$$Eh, V = 0.428 - 0.0022\,(T - 25)$$

where T = solution temperature, °C. Alternatively, select the value from Table 2580:I.

To determine the Eh of a sample relative to the standard hydrogen electrode, measure Eh of both sample and standard solution at the same temperature (i.e., ±0.1°C). Then calculate Eh value of the sample:

$$Eh_{system} = E_{observed} + Eh_{ZoBell\ reference} - Eh_{ZoBell\ observed}$$

where:

$E_{observed}$ = sample potential relative to reference electrode,
$Eh_{ZoBell\ reference}$ = theoretical Eh of reference electrode and Zobell's solution, relative to the standard hydrogen electrode (see Table 2580:I), and
$Eh_{ZoBell\ observed}$ = observed potential of ZoBell's solution, relative to the reference electrode.

The measurements described above can be applied analogously to other indicator electrode/reference electrode pairs and standard solutions.

b. Interferences: Specific interferences may be due to operation of either indicator or reference electrode, redox capacity or poise of the sample, sample preservation and handling, and temperature equilibration.

1) Sorption and poisoning effects on electrodes—Contamination of the electrode surface, salt bridge, or internal electrolyte in the case of reference electrodes, can lead to excessive drift, poor electrode response, and artifact potentials. Organic matter, sulfide, and bromide may cause these problems, particularly in long-term electrode use.[1,5-7] If excessive drift occurs or erratic performance of paired electrodes is observed in redox standard solutions after appropriate cleaning, refilling, or regeneration procedures, discard the faulty electrode and use a new one.

2) pH variations—Redox potential is sensitive to pH if hydrogen ion or hydroxide ion is involved in the redox half-cells. Cell potentials tend to increase as proton concentration increases (i.e., pH decreases) and Eh values drop as hydroxide concentrations increase (i.e., pH increase).

3) Sample handling and preservation—The sample poise will govern the resistance of the sample to change in redox potential; this phenomenon is analogous to the resistance to pH change afforded by buffer capacity. Except in concentrated process streams, sludges, leachates, and highly reducing or treated waters, the concentrations of oxidized or reduced species may be fairly low (i.e., $<10^{-4}M$). Under these conditions, handle reduced

TABLE 2580:I. POTENTIAL OF ZOBELL'S SOLUTION AS FUNCTION OF TEMPERATURE

T °C	E V	T °C	E V
1	0.481	16	0.448
2	0.479	17	0.446
3	0.476	18	0.443
4	0.474	19	0.441
5	0.472	20	0.439
6	0.470	21	0.437
7	0.468	22	0.435
8	0.465	23	0.432
9	0.463	24	0.430
10	0.461	25	0.428
11	0.459	26	0.426
12	0.457	27	0.424
13	0.454	28	0.421
14	0.452	29	0.419
15	0.450	30	0.417

samples very carefully to avoid exposure to atmospheric oxygen. A closed cell sampling configuration may be used.[4,8] Samples cannot be stored or preserved; analyze at sampling.

4) Temperature equilibration—Obtain Eh standard solution reading for the electrode pair at a temperature as close as possible to that of the sample. Temperature determines the Eh reference potential for a particular solution and electrode pair. It also may affect the reversibility of the redox reaction, the magnitude of the exchange current, and the stability of the apparent redox potential reading, particularly in poorly poised solutions. Hold temperature constant for all measurements and report it with Eh results.

2. Apparatus

a. pH or millivolt meter: Use a pH meter or other type of high-impedance potentiometer capable of reading either pH or millivolts (mV). For most applications, a meter scale readable to ± 1400 mV is sufficient.

b. Reference electrode consisting of a half-cell providing a constant electrode potential. See Section 4500-H$^+$.B.2*b*.

c. Oxidation-reduction indicator electrode: The platinum electrode is used most commonly. A noble metal or graphite electrode may be useful for specific applications.

1) Noble metal electrode—Noble metal (i.e., gold or platinum) foil, wire, or billet types of electrode are inert and resistant to chemical reaction. Clean and polish electrode surface to insure reliable performance. Platinum electrodes may be cleaned by strong acid soaking,[9,10] hydrogen peroxide and detergent washing,[11] and anodic activation.[10] Abrasive polishing with crocus cloth, jeweler's rouge, or 400 to 600 grit wet/dry carborundum paper may be best.[5]

2) Graphite electrode—A wax-impregnated graphite (WIG) electrode may be used, especially in aqueous suspensions or soils.[12,13] The WIG electrode is more resistant to electrode poisoning than electrodes made of platinum wire.

d. Beakers: Preferably use polyethylene, TFE, or glass beakers.

e. Stirrer: Use a magnetic TFE-coated stirring-bar-type mixer.

f. Flow cell: Use for continuous flow measurements and for poorly buffered solutions.

3. Reagents

a. Standard redox solutions: Standardize the electrode system against redox solutions that provide stable known Eh values over a range of temperatures. Although standard solutions are available, they do not cover the anticipated range of Eh values. Commercially prepared solutions may be used, particularly in field testing. The composition and Eh values of standard solutions are shown in Table 2580:II. With reasonable care, these solutions are stable for several months.

b. Eh electrode cleaners: Use either:

1) Aqua regia—Mix 1 volume conc nitric acid with 3 volumes conc hydrochloric acid. Prepare fresh and discard after use.

2) Chromic acid—Dissolve 5 g potassium dichromate, $K_2Cr_2O_7$, in 500 mL conc sulfuric acid.

4. Procedure

a. Instrument calibration: Follow manufacturer's instructions for using pH/millivolt meter and in preparing electrodes for use. Use a shorting lead to verify the zero point on the meter's millivolt scale. Equilibrate the standard solution to the temperature of the sample. Immerse electrodes in the gently stirred, standard solution in a beaker (or flow cell). Turn on meter, placing the function switch in the millivolt mode.

After several minutes for electrode equilibration, record reading to nearest millivolt. If the reading is more than ± 10 mV from the theoretical redox standard value at that temperature, replace reference electrode fluid and repeat the measurement. If that procedure fails to bring the reading to within ± 10 mV of the theoretical value, polish the sensing element of the indicator electrode with carborundum paper, crocus cloth, or jeweler's rouge. Rinse electrode thoroughly and recheck reading with a fresh portion of the standard solution. If the reading is

TABLE 2580:II. PREPARATION OF REDOX STANDARD SOLUTIONS

Standard Solution	Potentials of Pt Electrode vs. Selected Reference Electrodes at 25°C in Standard Solution					Weight of Chemicals Needed 1000 mL Aqueous Solution at 25°C
	Calomel	Silver:Silver Chloride Ag/AgCl			Standard Hydrogen	
	Hg/Hg_2Cl_2 saturated KCl	KCl 1.00*M*	KCl 4.00*M*	KCl saturated		
Light's solution[14]	+430	+439	+475	+476	+675	39.21 g ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ 48.22 g ferric ammonium sulfate, $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ 56.2 mL sulfuric acid, H_2SO_4, sp gr 1.84
ZoBell's solution[*,2]	+183	+192	+228	+229	+428	1.4080 g potassium ferrocyanide, $K_4Fe(CN)_6 \cdot 3H_2O$ 1.0975 g potassium ferricyanide, $K_3Fe(CN)_6$ 7.4555 g potassium chloride, KCl

* Store in dark plastic bottle in a refrigerator.

within ±10 mV of the theoretical value, record it and the temperature. If the reading is not within ±10 mV, repeat the cleaning procedure above or try another electrode. Then rinse the electrode with distilled water and proceed with the sample measurement.

Useful treatments for noble metal electrodes in restoring performance after long periods of use include immersion in warm (70°C) aqua regia for 1 to 2 min or 5 min in 6*N* HNO_3 after bringing to a boil. Alternatively treat with chromic acid solution followed by 6*N* HCl and rinse with water.

b. Sample analysis: Check system for performance with the standard solution, rinse electrodes thoroughly with sample water, then immerse them in the gently stirred sample. Let equilibrate, record Eh value to the nearest millivolt, and temperature to ±0.1°C. Repeat with a second sample portion to confirm successive readings within ±10 mV. Equilibration times vary and may take many minutes in poorly poised solutions. Successive readings that vary less than ±10 mV over 10 min are adequate for most purposes.

Make continuous flow or pumped sample measurements, particularly of poorly poised solutions, in a closed flow cell after external calibration of the electrode system. Recalibrate daily and more frequently if turbid, organic-rich, or high-dissolved-solids solutions are being measured.

See Table 2580:III for recommended combinations of electrodes, standards, and sample handling.

5. Trouble Shooting

a. Meter: Use a shorting lead to establish meter reading at zero millivolts whenever possible. If the meter cannot be zeroed, follow the manufacturer's instructions for service.

b. Electrodes: If the potentiometer is in good working order, the fault may be in the electrodes. Frequently, renewal of the filling solution for the salt bridge for the reference electrode is sufficient to restore electrode performance. Another useful check is to oppose the emf of a questionable reference electrode with that of the same type known to be in good order. Using an adapter, plug the good reference electrode into the indicator electrode jack of the potentiometer. Then plug the questionable electrode into the reference electrode jack. With the meter in the millivolt position, immerse electrodes in an electrolyte (e.g., KCl) solution and then into a redox standard solution. The two millivolt readings should be 0 ± 5 mV for both solutions. If different electrodes are used (e.g., silver:silver chloride versus calomel or vice versa), the reading should be 44 ± 5 mV for a good reference electrode.

Unless an indicator electrode has been poisoned, physically damaged, or shorted out, it usually is possible to restore function by proper cleaning.

TABLE 2580:III. RECOMMENDED COMBINATIONS FOR SELECTED SAMPLE TYPES

Sample Type	Indicator Electrode(s)	Reference Electrode	Type of Sample Cell
Process stream (low Br^-) (S^{2-})	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)
(high Br^-)	Pt or Au	Calomel or silver: silver chloride with salt bridge (double junction reference electrode)	
Natural waters			
Surface waters	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode) or beaker
Groundwater	Pt or Au	Calomel or silver: silver chloride	Closed continuous flow (dual indicator electrode)
Soils, sludges	WIG, Pt wire	Calomel or silver: silver chloride	Beaker or soil core

6. Calculation

$$Eh_{system} = E_{observed} + E_{reference\ standard} - E_{reference\ observed}$$

Report temperature at which readings were made.

7. Precision and Bias

Standard solution measurements made at stable temperatures with a properly functioning electrode system should be accurate to within ±10 mV. Calibration precision as reflected by the agreement of dual platinum electrodes versus an Ag:AgCl reference electrode for over a 2-year period has been estimated at ±15 mV (i.e., one standard deviation) in ZoBell's solution (N = 78) at approximately 12°C. Precision on groundwater samples (N = 234) over the same period has been estimated at ±22 mV (i.e., ± one standard deviation) in a closed flow cell.[15]

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APPENDIX A-7

Procedure for Non-Purgeable Organic Carbon: Method 415.1

Total Organic Carbon - Method 415.1 with Dohrmann DC-190

1.0 Procedure

Perform Total Organic Carbon analysis in accordance with "Organic Carbon, Total", Method 415.1 (Combustion or Oxidation) and in accordance with chapters 6 and 10 of the operating manual for the Dohrmann DC-190 high temperature organic carbon analyzer as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

ORGANIC CARBON, TOTAL

Method 415.1 (Combustion or Oxidation)

STORET NO. Total 00680
Dissolved 00681

1. Scope and Application
   1.1 This method includes the measurement of organic carbon in drinking, surface and saline waters, domestic and industrial wastes. Exclusions are noted under Definitions and Interferences.
   1.2 The method is most applicable to measurement of organic carbon above 1 mg/1.
2. Summary of Method
   2.1 Organic carbon in a sample is converted to carbon dioxide (CO_2) by catalytic combustion or wet chemical oxidation. The CO_2 formed can be measured directly by an infrared detector or converted to methane (CH_4) and measured by a flame ionization detector. The amount of CO_2 or CH_4 is directly proportional to the concentration of carbonaceous material in the sample.
3. Definitions
   3.1 The carbonaceous analyzer measures all of the carbon in a sample. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to analysis dictates the definition of the carbon as it is measured. Forms of carbon that are measured by the method are:
      A) soluble, nonvolatile organic carbon; for instance, natural sugars.
      B) soluble, volatile organic carbon; for instance, mercaptans.
      C) insoluble, partially volatile carbon; for instance, oils.
      D) insoluble, particulate carbonaceous materials, for instance; cellulose fibers.
      E) soluble or insoluble carbonaceous materials adsorbed or entrapped on insoluble inorganic suspended matter; for instance, oily matter adsorbed on silt particles.
   3.2 The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment of the sample and instrument settings defines the types of carbon which are measured. Instrument manufacturer's instructions should be followed.

Approved for NPDES
Issued 1971
Editorial revision 1974

4. Sample Handling and Preservation

4.1 Sampling and storage of samples in glass bottles is preferable. Sampling and storage in plastic bottles such as conventional polyethylene and cubitainers is permissible if it is established that the containers do not contribute contaminating organics to the samples.
NOTE 1: A brief study performed in the EPA Laboratory indicated that distilled water stored in new, one quart cubitainers did not show any increase in organic carbon after two weeks exposure.

4.2 Because of the possibility of oxidation or bacterial decomposition of some components of aqueous samples, the lapse of time between collection of samples and start of analysis should be kept to a minimum. Also, samples should be kept cool (4°C) and protected from sunlight and atmospheric oxygen.

4.3 In instances where analysis cannot be performed within two hours (2 hours) from time of sampling, the sample is acidified ($pH \leq 2$) with HCl or H_2SO_4.

5. Interferences

5.1 Carbonate and bicarbonate carbon represent an interference under the terms of this test and must be removed or accounted for in the final calculation.

5.2 This procedure is applicable only to homogeneous samples which can be injected into the apparatus reproducibly by means of a microliter type syringe or pipette. The openings of the syringe or pipette limit the maximum size of particles which may be included in the sample.

6. Apparatus

6.1 Apparatus for blending or homogenizing samples: Generally, a Waring-type blender is satisfactory.

6.2 Apparatus for total and dissolved organic carbon:

6.2.1 A number of companies manufacture systems for measuring carbonaceous material in liquid samples. Considerations should be made as to the types of samples to be analyzed, the expected concentration range, and forms of carbon to be measured.

6.2.2 No specific analyzer is recommended as superior.

7. Reagents

7.1 Distilled water used in preparation of standards and for dilution of samples should be ultra pure to reduce the carbon concentration of the blank. Carbon dioxide-free, double distilled water is recommended. Ion exchanged waters are not recommended because of the possibilities of contamination with organic materials from the resins.

7.2 Potassium hydrogen phthalate, stock solution, 1000 mg carbon/liter: Dissolve 0.2128 g of potassium hydrogen phthalate (Primary Standard Grade) in distilled water and dilute to 100.0 ml.
NOTE 2: Sodium oxalate and acetic acid are not recommended as stock solutions.

7.3 Potassium hydrogen phthalate, standard solutions: Prepare standard solutions from the stock solution by dilution with distilled water.

7.4 Carbonate-bicarbonate, stock solution, 1000 mg carbon/liter: Weigh 0.3500 g of sodium bicarbonate and 0.4418 g of sodium carbonate and transfer both to the same 100 ml volumetric flask. Dissolve with distilled water.

7.5 Carbonate-bicarbonate, standard solution: Prepare a series of standards similar to step 7.3.

NOTE 3: This standard is not required by some instruments.

7.6 Blank solution: Use the same distilled water (or similar quality water) used for the preparation of the standard solutions.

8. Procedure

8.1 Follow instrument manufacturer's instructions for calibration, procedure, and calculations.

8.2 For calibration of the instrument, it is recommended that a series of standards encompassing the expected concentration range of the samples be used.

9. Precision and Accuracy

9.1 Twenty-eight analysts in twenty-one laboratories analyzed distilled water solutions containing exact increments of oxidizable organic compounds, with the following results:

Increment as TOC mg/liter	Precision as Standard Deviation TOC, mg/liter	Accuracy as Bias, %	Accuracy as Bias, mg/liter
4.9	3.93	+15.27	+0.75
107	8.32	+ 1.01	+1.08

(FWPCA Method Study 3, Demand Analyses)

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SECTION 6

OPERATION

INTRODUCTION

This section contains instructions for routine operation along with detailed descriptions on how to operate and calibrate the different modes.

6.1 ROUTINE OPERATION

SUMMARY
* Daily Start-Up * Daily Operation * Daily and Long-term Shutdown

DAILY START-UP

Check utility supply.	Enough carrier gas for a day's operation. Acid reservoir at least 1/3 full.
Replenish IC chamber.	Confirm the IC chamber is half full (gas off). Fill the IC chamber by using the "Acid to IC chamber" function (press **MAIN 2 5**). Each use of this function will result in 20 pulses and is equivalent to 2 ml of acid.
Turn on gas.	Press **CARRIER** .
For Boat Users :	Connect the 1/8 inch PTFE line from the boat module furnace to the DC-190 dehumidifier (see Figure 4.8).
Check system status.	(Press **MAIN 1** to view the status menu.) Flow rate = 180 - 220 cc/min. Dryer temperature = 0 - 10 ^{O}C Furnace Temperature = Furnace set point (Furnace light is green.) For most applications, the temperature should be 680^{O}C. Confirm or change set-up number on display (see Section 6.8).
Check set-up.	(See Section 6.2 for help in choosing set-up.) Modes last used are lit up. Make any changes for the day and print the set-up parameters. System is ready for analysis.

DAILY OPERATION

Press START when ready.

It is good practice to run a check standard at the beginning of the day before analyzing unknowns, especially if any conditions have been changed. Update calibration if needed. See Section 6.3 for notes on operating and calibrating.

DAILY SHUTDOWN

Check the RUN status.

The unit should not be in a RUN mode.

For Boat Users :

Disconnect the 1/8 inch PTFE line which runs from the boat furnace to the dehumidifier.

Shut off the gas.

Press CARRIER .

NOTE: The furnace and the NDIR should be left on unless the unit is going to be relocated or will not be used for a long time. Frequently turning the furnace on/off reduces the life of the heater element. The NDIR requires at least 2 hours for stabilization after power up.

6.2 SELECTING THE ANALYSIS PARAMETERS

Most analysis have three parameters:

1) Analysis mode.
2) Inlet mode.
3) Volume.

NOTE: The ASM and RSM operating modes have other parameters which must be selected. See Sections 6.4 and 6.5 for guidelines in selecting these parameters.

SELECT THE ANALYSIS MODE

Use Table 6.1 to match your application to an analysis mode. The default mode is **NPOC**. To set another mode, press the corresponding button.

Table 6.1
ANALYSIS MODE SELECTION

ANALYSIS MODE	APPLICATION	METHOD
NPOC	Any water sample.	IC purged from sample at sparging station. Inject into TC port. NPOC $\xrightarrow[\text{Furnace}]{\text{TC}}$ CO_2
TOC	Any water sample. Method of choice when sample has no volatiles.	TOC = TC - IC Two (2) injections per analysis. DC-190 calculates the difference. See See TC and IC descriptions.
IC	Any sample where dissolved CO_2 or carbonate concentrationis of interest.	Sample injected into IC port. IC $\xrightarrow[\text{Chamber}]{\text{IC}}$ CO_2
TC	Any water sample.	Sample injected into TC port. TC $\xrightarrow[\text{Furnace}]{\text{TC}}$ CO_2
POC	Water sampler where volatile organics or other purgeables are of interest.	Sample is sparged at POC sparge station. LiOH scrubber removes IC from sparged gas. POC Gas --> IC Scrubber --> POC $\xrightarrow[\text{Furnace}]{\text{TC}}$ CO_2
Boat Option, TC	Solids, sludges, slurries and waters with particulates greater than 0.5 mm.	Sample introduced onto platinum boat. Boat pushed into 183 furnace. Sample $\xrightarrow{800^{\circ}C}$ CO_2

SELECT THE INLET MODE

The default inlet mode is **SYRINGE** . To select a different mode, refer to the following Table, then press the button corresponding to the new inlet mode.

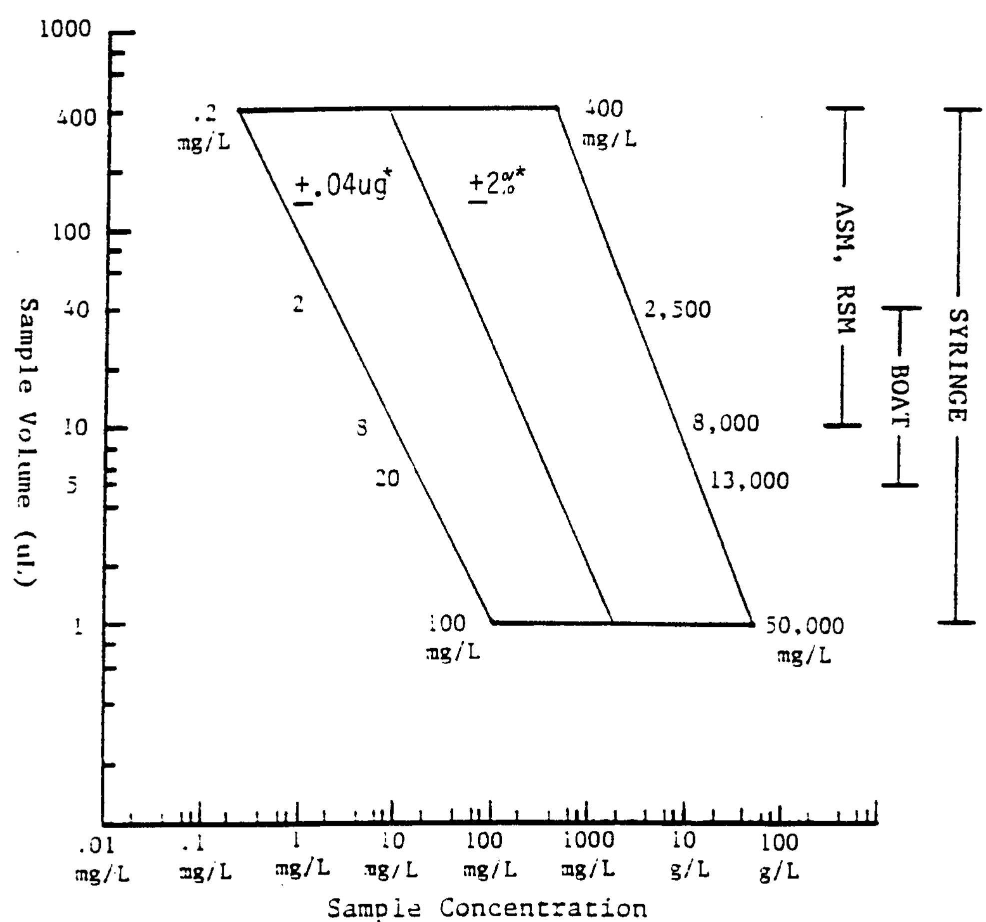
Table 6.2
INLET MODE SELECTION

ANALYSIS MODE	INLET MODE	DEFAULT Volume (ul)	DEFAULT Range (mgC/L)	POSSIBLE VOLUME (ul)
NPOC TOC IC TC	Syringe	50	1 - 2000	1 - 400 20 - 200 *
	ASM	50	1 - 2000	10 - 400
TOC IC TC	RSM	50	1 - 2000	10 - 400
TC NPOC	Boat	40	2 - 4000	5 - 40
POC	N/A	10 mL	.01 - 20	2 - 10 mL

* This is the range for the manual micropipettor which is used with the **SYRINGE** mode.

SELECT VOLUME

The default volume and corresponding concentration range for each inlet mode are shown in the previous table. If the default concentration range is unsuitable, a better sample volume may be selected using Figure 6.1. Enter the new sample volume on the inlet mode menu.



* Expected precision. See Section 1.4.

FIGURE 6.1 Concentration Range vs. Sample Volume

EXAMPLE: Expected sample concentration range = 5 to 5,000 ppm.
From Figure 6.1, 20 ul gives 4 to 6,000 ppm.
(Note the logarithmic scales.)
20 ul is compatible with all inlet modes, except POC.

6.3 MANUAL OPERATION

Use these instructions for syringe or micropipettor operation in NPOC, TOC, IC, TC or POC modes. The following table shows the general operation sequence for syringe injections. Specific notes for each analysis mode follow the table.

GENERAL OPERATION SEQUENCE - ALL MODES
* If it is desired to save the current operating parameters before making any changes, select a new set-up number (see Section 6.7). * Choose set-up. * Have the syringe filled and ready. (Have the septum installed as shown in Figure 6.2.) * Press **START** . * Inject the sample. (Review the injection technique for the mode selected.) * At the conclusion of the analysis, the screen will display the final ppm value along with: **Continue Y/N ?** (This question must be answered before the system will perform any other action.) * Press **YES** to make more injections. * Press **NO** to end the run. * Press **STOP** to end the run after the current analysis. To terminate the run, immediately press **STOP** five times.

ABOUT SYRINGES

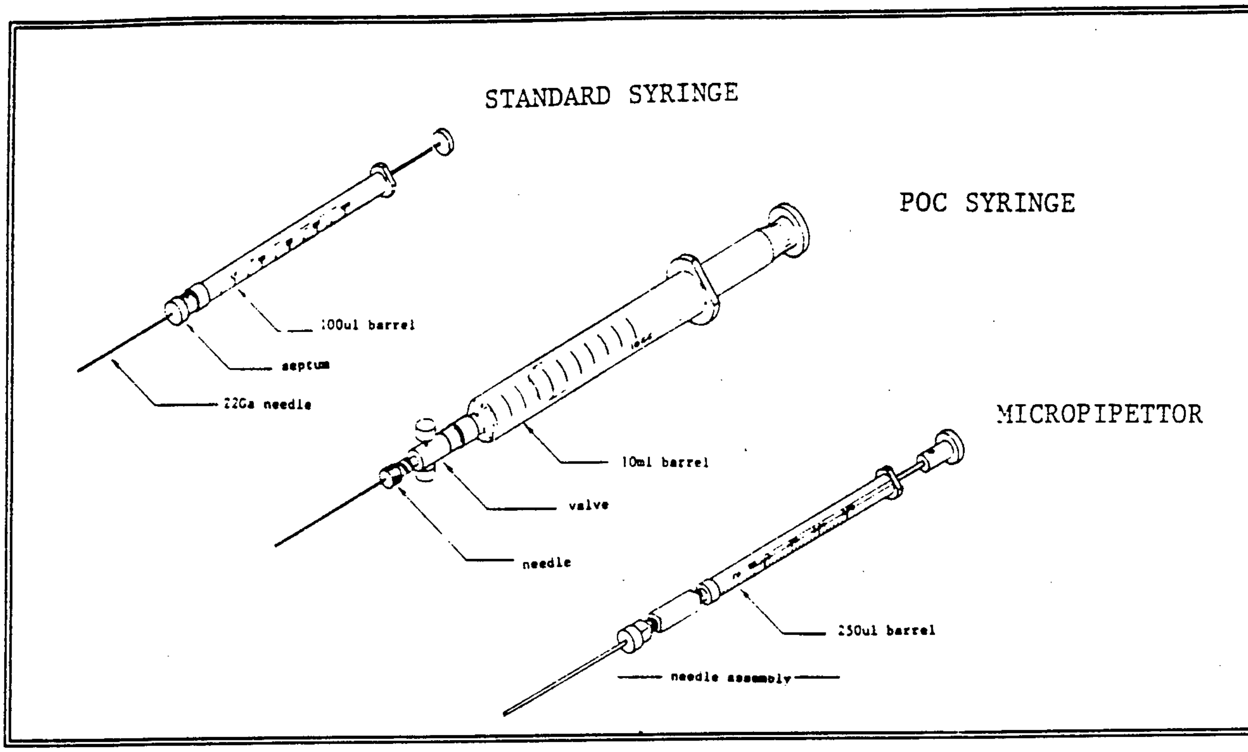


FIGURE 6.2. SYRINGE ASSEMBLIES

Assemble the syringes and micropipettor as shown in Figure 6.2. Always have a grey septum attached to the syringe or pipettor.

It is important for reliable sample introduction to use blunt-point needles such as those supplied with the DC-190. Side-port needles should not be used except on the POC syringe.

The 100 uL syringe (P/N 060-871) provided with the DC-190 has a 22S gauge (0.006 inch I.D.) needle. The 22 gauge (0.016 inch I.D.) replacement needles (P/N 060-872) are provided in the DC-190 operating kit for sample types requiring a larger I.D. needle.

Also available are a micropipettor barrel (250 uL syringe barrel, P/N 060-875) and a micropipettor needle (P/N 888-297). The micropipettor is used for samples containing particulates up to 0.5 mm diameter or samples which are incompatible with (react with or corrode) a stainless steel needle. The micropipettor probe should be used with a 250 uL syringe barrel only.

Injection Technique

As soon as the INJECT light comes on, press **OPEN/CLOSE**

Immediately insert the syringe into the injection port that has the illuminated LED.

Make seal during injection by pressing the grey septum against the port.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment

Inject at 50 ul/sec rate.

Withdraw the syringe and immediately press **OPEN/CLOSE** to close the port.

For 1 - 10 ul volumes, wait 5 seconds in between injecting and withdrawing syringe.

Micropipettor Users:

When using a micropipettor, wipe off the outside of the probe after drawing up the sample.

For volumes below 50 uL, the injection rate is crucial to obtaining reproducible results. Make the injection rapidly without jarring the syringe. (**HINT:** After withdrawing the syringe, look at the tip. If it is wet on the outside, inject faster; if it is partially empty, inject slower.)

Wait 10 seconds after injecting before withdrawing the pipettor for all volumes.

Sample Pretreatment

None, unless the samples are inhomogeneous or contain large particulates (> 0.5 mm diameter).

TOC

(This is a combination of the TC and IC modes.)

Injection Technique Use the same technique as for the TC and IC modes.

Make two injections per analysis.

The first injection goes in the TC port.

Have the syringe filled and ready for the second injection which is made to the IC port. Look for the prompt from the display.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment

NOTE: When high pH samples are expected, treat combustion tube with 2 injections of 100 ul of pH1 HCl or HNO_3 solution.

NPOC

(This is the default analysis mode.)

Injection Technique

Use the same technique as for the TC and IC modes.

Inject into the TC port only.

Samples will expand rapidly when injected into the combustion tube. Hot steam may vent from the injection port unless a good seal is made with the syringe septa when injecting.

The DC-190 has a 100% O_2 atmosphere in the combustion tube. Samples with more than 10% hydrocarbons may explode when injected into this environment.

Sample Pretreatment

The sample must be sparged prior to injection to remove the IC.

To sparge the sample:

- Pour about 10 mL of sample into a 20 mL vial (P/N 889-726).
- Screw the vial into Sparger A or Sparger B.
- Press A or B, and then 1 to start sparging.
- The sample will be automatically acidified. Each unit of "Add acid" is equivalent to 100 ul.
- Sparging will stop automatically at the end of sparge time.
- Remove the vial and cap it until the analysis is run.

Two samples can be sparged simultaneously.

Samples containing large particulates (> 0.5 mm) must be pretreated as directed in Section 10.2.

POC

Priming and sparging steps involve acid pumping into the appropriate vessels. Make sure the plumbing is properly connected to avoid acid injury to persons or property.

Injection Technique

As soon as the INJECT light comes on, inject the sample into the POC sparger through the injection port.

When the analysis is over, withdraw the remaining sample from the sparger with the syringe.

Sample Pretreatment

None.

How to Fill the Syringe

Remove the plunger from the syringe and close the syringe valve and needle. Open the sample or standard container, which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 10 ml.

This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample container, the analyst should fill a second syringe at this time in case the first analysis is unsuccessful.

6.4 AUTOSAMPLER OPERATION

INTRODUCTION

The DC-190 Autosampler (ASM) option is designed for unattended operation for many hours. The sample tray holds 32 8 mL vials. Automatic acid addition and sparging are provided by the sparge tower to remove inorganic carbon for NPOC analysis. The sample probe may be rinsed with either water and/or sample between analyses. The ASM can handle samples with particulates up to 0.5 mm and the sample may be stirred with gas before the sample is drawn to insure uniform sampling. Cross-contamination is minimized by the use of non-wetting materials for all sample contacting parts. Sample vials may be marked as blanks or standards for automatic calibration of the system during the ASM run.

The ASM offers an autoranging capability which will adjust the sample volume to maintain the peak integral within the range of the detector. Since the dynamic range of the DC-190 system is very wide (10,000 to 1), activation of the autoranging will normally be a very rare event. When this feature is active, the DC-190 will check the first replicate of a vial in the ASM mode to verify that the peak integral is within range. If the peak integral is below range, the result will be printed, but ignored in future statistical calculations. The injection will then be repeated, but with a volume 5 times larger than the original injection. If the peak integral is over range, a similar procedure is followed with a volume one fifth the original volume. The volume adjustment will be repeated until the peak integral is within range. If an adjustment would result in a volume outside the 10 to 400 uL range, the volume will be set to either 10 or 400 uL as appropriate and no further adjustment will be made. The original injection volume will be restored at the beginning of the next sample vial. The accuracy of the autoranged data may suffer somewhat because the ASM was not calibrated with the new volume. The inaccuracy without autoranging is potentially much worse, however, than with autoranging. If desired, the results of autoranged data may be rechecked later.

Below is a table of expected and observed volumes for the ASM. These values are approximate and will vary from instrument to instrument. This volume variation only affects autoranged data. This will not apply to normal calibrated ASM data because the same volume is used for analysis.

VOLUME (uL)	
Expected	Observed
10	10.3
20	19.5
40	35.4
80	70
100	92
200	194
400	400

OPERATION

* Refer to DAILY START-UP in Section 6.1 to prepare the analyzer for operation.

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Refer to Section 6.2 and select the analysis mode and volume desired. See Table 6.3 for guidelines to set the other operating parameters.

* Place the vials in the sample tray beginning with tray position 1. Refer to Table 6.4 and mark the vials as blanks, standards, or samples as appropriate. Mark the first empty tray position after the samples as indicated in Table 6.4 to terminate the run.

* Clean and fill the rinse bottle with DI water if water rinses were called for on the Rinse\stir menu.

* Check that the acid bottle is at least 1/3 full of acid solution if set up for NPOC analysis.

* Check that the printer is ready and has sufficient paper.

* Press **START**.

* There are two ways to end the run before completion. Press **STOP** to end the run after the current analysis. To terminate the run immediately, press **STOP** five times. After an immediate bail out, the ASM may have to be returned to its resting position. The sparge arm may be raised by selecting "Raise sparge arm" (**1**) on the "Sparge arm menu" (**MAIN 2 5 3 3**). The sample arm may be returned to the rinse bottle position by selecting "Move arm to rinse" (**4**) on the "Sample arm" menu (**MAIN 2 5 3 2**). Always check the "Furnace/IC ports" menu (**MAIN 2 5 5**) to be sure the inlet ports are shut (even if the indicator lights next to the ports are not lit).

TABLE 6.3
ASM OPERATION PARAMETER GUIDELINES

# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats, with 3 being the default.
Sparge time (min)	The default time (3 minutes) should be satisfactory for almost all samples as long as the pH is in the proper range (see "Acid volume" below). This option is applicable to the NPOC mode only.
Acid volume	The pH must be adjusted to a value less than 4. It may be necessary to check a few samples after acid addition and make adjustments by trial and error until the acid addition matches the particular samples being analyzed. The default is 1 (each unit of acid volume is equivalent to 100 ul). This option is applicable to the NPOC mode only.
The following selections are on the "Rinse and /or stir" menu:	
# of rinses w/water	This option specifies the number of times the ASM sample probe and loop will be rinsed with water between each vial.
# of rinses w/sample	Similar to the above option except that the ASM will rinse with sample before the first injection from each vial.
Sample stir time (sec)	Specifies the time that the sample will be stirred before the sample is drawn into the sample loop. The allowed range is 0 - 30 seconds (default = 0). In most applications, 15 seconds will provide effective stirring. Stirring is accomplished by bubbling gas out of the sample probe to suspend particulates and obtain a more uniform sample.
Auto-range	When set to "Yes", the DC-190 will automatically adjust the injection volume. "No" is the default setting. See the INTRODUCTION to this section for details on this feature.
CG off after	The default "No" means the carrier gas (CG) will not be turned off at the end of an ASM run. A "Yes" will cause the carrier gas to be turned off 10 minutes after the end of an ASM run. During this period, the red light in the **START/STOP** button will blink as if the run is still in progress.

TABLE 6.4
ASM VIAL MARKERS

VIAL	PEG POSITION INNER*	PEG POSITION OUTER**	INDICATION
No	No	No	Skip Position
Yes	No	No	Sample
Yes	Yes	No	Blank
Yes	No	Yes	Standard
Yes	Yes	Yes	Rinse Sample***
No	No	Yes	Terminate Run

* Peg hole closer to center of sample tray.
** Peg hole closer to sample vial.
*** Sample is used for rinse only (no analysis).

NOTE: If the printer runs out of paper or jams during a run "Print last run" (**MAIN 2 3**) will reprint the run data from a buffer. This allows data otherwise lost to be retreived. The buffer which retains the data is not large enough, however, to hold a complete run of data in all cases. This buffer has sufficient capacity to hold data from approximately 32 vials with 3 replicates per vial in modes where each replicate requires one line to print (TC, IC, or NPOC). In the TOC mode, each replicate requires three lines to print. In this mode, the buffer will only hold approximately 10 vials with 3 replicates per vial. The buffer is filled on a first in first out basis so that the data remaining at the end of the run will be the last data point back until the buffer is full.

6.5 OPERATION OF THE RSM OPTION

The RSM option allows the continuous sampling of a sample stream which is tapped to flow through the RSM sample cell. The ASM will perform the designated number of replicates on the sample stream and then wait for a designated time period. The sampling cycle is then repeated. The TC, IC, and TOC analysis modes may be performed using the RSM option. However, if the sample stream IC and TC levels are not constant, the accuracy of the TOC analysis may suffer due to the time lag between the IC and TC portions of the analysis.

* Adjust the sample flow rate to the sample cell by slowly opening the needle valve (counter clockwise) until the water level stabilizes slightly above the drain port of the sample cell.

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).

* Select **TC**, **IC**, or **TOC** (see Section 6.2 for selection guidelines) and then **RSM** to set the analysis mode. Verify that the operating parameters are set to the desired values. Use the guidelines in Table 6.5.

* Calibrate the DC-190 according to the RSM calibration procedure in Section 6.8.

* Press **START** to begin the analysis. The RSM will continue until manually stopped.

* To stop the analysis, press **STOP** (same button as **START**). This will stop the DC-190 at the end of an analysis in progress or immediately during the time between runs. To stop the run immediately during an analysis, press the **STOP** button 5 times.

TABLE 6.5

RSM OPERATING PARAMETER GUIDELINES

Sample volume	See Figure 6.1
# of repeats	Select a number that is statistically comfortable. The allowed range is 1 - 5 repeats with 3 being the default.
Time between runs	This is the time from the conclusion of the last replicate of a group to the beginning of the first replicate of the next group. The allowable range is 0 to 54 minutes with a default of 0 minutes.

6.6 OPERATION OF THE BOAT OPTION

Use the boat sampler for slurries, sludges, solids, and suspensions. Operate in either the **TC** or **NPOC** mode. Refer to "Installation and Operation of the 183 Boat Sampling Module" (P/N 915-240) for sample introduction instructions (Section V, Parts 5A and 5B). The DC-190 calculates ppmC from liquids or solids.

SAMPLE TYPE	SAMPLE INTRODUCTION	CONCENTRATION UNITS
Liquids, light slurries, suspensions	See 183 Instructions for **Liquids**	mg/L
Solids, heavy slurries	See 183 Instructions for **Solids**	ug/g

* If it is desired to save the current operating parameters before making any changes, select a new Set-up number (see Section 6.8).
* Press **BOAT TC** or **NPOC** .
* Press **1** until the appropriate units are displayed.
* Introduce the sample into the boat - see "Installation and Operation of the 183 Boat Sampling Module".
* Press **START** and follow the 183 instructions.
* If ug/g units are selected, enter the sample weight when asked - "Sample weight (mg)?".
* **SOLIDS ONLY:** Enter the sample weight when asked - "Sample weight (mg)?".

6.7 CALIBRATION

The DC-190 offers a choice of either one point or two point calibration. Two point calibration is equivalent to subtracting the blank value automatically. The DC-190 system always calculates a two point linear calibration. If only a single point calibration is desired, the System Blank may be set to 0 before updating the Calibration Factor. In this case the System Blank will remain 0 after updating the Calibration Factor resulting in a single point calibration. Since the system blank for IC is normally insignificant, its value is set to zero and IC analysis always has one point calibration. When two-point calibration is used, both calibration factor and system blank are recalculated each time either the calibration factor or system blank is updated. In TOC mode, the system uses TC value for calibration and blank update.

The DC-190 system provides a common calibration set (calibration factor and system blank) for SYRINGE, ASM, and RSM modes. POC and BOAT modes have their own calibration sets. When changing inlet mode from SYRINGE to ASM or RSM, calibration stays the same. When changing inlet mode from SYRINGE, ASM, or RSM to POC or Boat, calibration changes accordingly. The multiple set-up function (see Section 6.8) provides capability to store and retrieve up to 5 calibration sets.

Since SYRINGE and ASM/RSM calibrations are not necessarily the same, calibration for these modes should be done separately. Use the multiple set-up function to store the different calibration sets.

SUMMARY
1. System Blank 2. Calibrating Syringe, POC, or Boat Modes 3. Calibrating The ASM Mode 4. Calibrating The RSM Option 5. Omitting Outlier Data 6. Calibration Equations

SYSTEM BLANK

System blank is defined as the response contributed by the analyzer when carbon-free water sample is injected and analyzed. In reality, it is very difficult to produce and preserve the carbon-free water. Thus the true system blank and the carbon content of the water sample cannot be accurately distinguished. However, the carbon content of high purity water can be below the defection limit (.2ppmC) and the response with such water may be assumed as the system blank. When it exists, the blank value is subtracted from every analysis except in IC mode where blank is always assumed to be zero.

The system blank becomes increasingly important for analyses below 10 mgC/L as shown :

MODE	VOLUME	TYPICAL BLANK (mgC/L)
TC NPOC	400 ul	.10 - .40
IC	400ul	0*
POC	10ml	0 - .03
BOAT	40ul	2.0 - 4.0

Factors affecting the blank :

- Cleanliness of syringes, spargers and IC chamber.
- Sample handling.
- Age and sample history of TC and boat combustion tubes.
- Dehumidifier temperature.

CALIBRATING THE SYRINGE, POC, or BOAT INLET MODES

See "SYSTEM BLANK" earlier in this section for guidelines to determine whether a two point calibration is needed for the samples to be analyzed.

* Analyze a standard in the analysis mode to be used. An average of at least two determinations is recommended. Respond **NO** to the prompt "Continue yes/no?" when satisfied with the results.

* Outlier data can be omitted at this point if desired. See the section "OMITTING OUTLIER DATA" at the end of this section for details on how to do this.

* Press **CALIBRATE** to review the calibration menu :

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.

* Press **5** to update the Calibration Factor. The new calibration factor will be calculated and displayed on the menu.

* To complete a two point calibration, if desired, repeat the above procedure with a blank sample. Use the cleanest reagent water available (less than 0.150 mgC/L). Press **6** to update the System Blank.

* The DC-190 is now calibrated for the selected analysis mode.

Analyze a check standard with each sample set. If the reported value deviates from the expected value by more than 2%, re-calibrate the system.

Note To Boat Users: It is easy to use a liquid standard to calibrate the DC-190 even when using "ug/g" units to analyze solid samples. For example, to obtain 10 mg of sample, simply inject 10 ul of standard. This relationship holds as long as the density of the standard is 1 g/mL, which will be true for most water-based standards.

CALIBRATING THE ASM INLET MODE

* Select the ASM operating paramaters as described in Section 6.4 and press **START** to begin analyzing the standard.

* Place the vials of standard in the first tray positions. It is recommended that two vials of standard be placed next to each other at the beginning of the ASM sample tray. Place a peg in the outer hole next to the second vial to mark it as a standard for calibration (see Table 6.4).

* If blanks are to be determined, place two or three vials of blank immediately following the vials of standard. In most circumstances, two vials are sufficient. For best accuracy at low levels, three vials are recommended. Place a peg in the inner hole next to the last of the two or three blank vials to instruct the DC-190 to determine a new blank value (see Table 6.4).

* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If a one point calibration (no subtraction of the blank) is desired, make sure the System Blank is set to 0. Make any necessary changes.

* Place the sample vials in the sample tray following the standard and blank vials, and run the analysis according to the operation instructions in Section 6.4. The DC-190 will automatically calculate and use the calibration factor and blank value.

CALIBRATING THE RSM OPTION

The RSM mode is easiest to calibrate using a vial of the desired standard rather than by pumping the standard through the RSM sample cell. This method is described in the following steps:

* Lift the sample cell from its holder and secure it in the clip located to the left of the black cell holder.

* Place an ASM vial (P/N 080-140) containing the standard solution into the black cell holder.

* Select the RSM operating paramaters as described in Section 6.5 and press **START** to begin analyzing the standard.

* Since the RSM does not stop automatically, it is necessary to manually stop it by pressing **STOP** (the same button as **START**) during the last desired replicate of the standard. The DC-190 will then stop at the end of the current analysis.

* Outlier data can be omitted at this point if desired. See the Section "OMITTING OUTLIER DATA" at the end of this Section for details on how to do this.

* Press **CALIBRATE** to review the calibration menu:

1. Calibration factor	1
2. System blank	0
3. Sample size	50
4. Std. concentration	1000
5. Update cal-factor	
6. Update system blank	
7. Other actions	

* Verify that the sample size and standard concentration shown on the "Calibration" menu are correct. If subtraction of the blank is not desired, make sure the System Blank is set to 0. Make any necessary changes.

* Press **5** "Update cal factor" to calculate and store a new calibration factor.

* Repeat the above procedure with a blank sample and press 6 "Update system blank" on the "Calibration" menu if an update of the system blank is desired.

OMITTING OUTLIER DATA

The DC-190 provides the ability to reject outlier data when operated in the manual modes (Syringe, Boat, and POC) and the RSM mode (no provision for outlier rejection is made in the ASM mode). A new average and standard deviation are calculated after the data is rejected. This feature saves having to re-run a data set due to a bad data point when updating the Calibration Factor or System Blank. The DC-190 will not allow the number of replicates to be reduced to less than 2 as a result of data rejection. Data rejection is accomplished by the following steps:

* Complete the run by responding **NO** in one of the manual modes or **STOP** in the RSM mode (see the calibration instructions for the mode in use) to the prompt at the end of the analysis. Three or more replicates must have been generated.

* Select the "Auxiliary functions" menu (**MAIN 2**) and press **1** "Omit an outlier".

* At the prompt, enter the number of replicates to reject. Each replicate to be rejected will be prompted for separately. Enter a replicate number after each prompt.

* New statistics will be displayed on the screen and printer. An update of the Calibration Factor or System Blank will now be based on the new average value.

* If the "Omit an outlier" menu item is selected again after the current data set has been edited, the DC-190 will start the data rejection over and ignore the previous data editing.

CALIBRATION EQUATIONS

The following equations are used in the DC-190 system.

The equation for determining a calibrated result is:

$$y = (Fx - b) / V$$

where:

y	=	Concentration (calibrated) of sample.
x	=	NDIR peak with background subtracted. Normally invisible to the user. The displayed value, y, may be made to equal x by setting F, b, and V to the appropriate values (1, 0, and 1, respectively)
F	=	"Calibration Factor". This is the slope of the linear fit line.
b	=	Intercept. This is an internal parameter which is invisible to the user.
SB	=	"System Blank" = b/V.
V	=	Sample volume (or mass).

The quantities F and SB are the ones displayed on the calibration menu and are the ones which can be edited directly.

The Calibration Factor and Blank are calculated by:

$$F_n = F_o (C_s / y_s)$$

$$b_n = b_o (F_n / F_o)$$

where:

C_s	=	Concentration of the standard.
o	=	Old value.
n	=	New value.
s	=	Value for Standard.

These are the equations used internally by the DC-190 system. Both Fn and bn are re-calculated each time either the Calibration Factor or the System Blank is updated. It should be noted that if the old value bo is already 0, the new value bn and therefore SB will also be 0. This provides a means to have the system effectively do a one point calibration update when it calculates a new Calibration Factor. These equations may also be used to manually calculate the values and enter them on the "Calibration" menu directly.

6.8 USING THE MULTIPLE PARAMETER SETS

The DC-190 provides the capability to store 5 complete sets of operating parameters. This capability allows the user to return to a previously defined set of operating parameters without having to re-enter the parameters. The parameter set includes the inlet mode, the analysis mode, the parameters appropriate to the analysis/inlet mode as well as the Calibration Factor and System Blank..

One of the parameter sets is always the "working" set-up. This is the parameter set associated with the current set-up number. Any run started will now use the parameter values currently contained in the working parameter set. As changes are made to the operating parameters, these changes are made to the working set-up.

When a new set-up number is selected, the parameter values in the previous set-up are saved as they were at the time of the new selection. The working parameter set now takes the values associated with the new set-up number. Any run started will use the new parameter values and any parameter changes are now made to the new parameter set.

Returning to the previous set-up number will restore the operating parameters to the state they were in when the set-up number was last used.

If it is desired to save the current set of parameter values for future re-use, a new set-up number should be selected before starting to define a new parameter set.

To determine the set-up number:	Display the "System status" menu (**MAIN 1**) . Line 5 "Analysis set-up" indicates the current Set-up number.
To change to another set-up number:	Select the "System status" menu (**MAIN 1**) and then "Analysis set-up" (**5**) and enter the new Set-up number. This saves the current parameter set.

To print the current parameter set: Press the analysis mode button with the lit LED and then select the "Print set-up" option on the displayed operating parameter menu.

To print all the parameter sets: Display the "System status" menu (**MAIN** **1**). Press **6** "Print set-up selections".

USING THE CLIPBOARD

A clipboard is provided in the DC-190 system which allows the Calibration Factor and System Blank to be copied from one parameter set to another. This feature can save time and effort when changing from parameter set to another after the system has been calibrated. Use the following steps:

* Select the "Other actions" section of the "Calibration" menu (**CALIBRATE 7**).

* Verify that the "Analysis set-up" shown on line 4 is the one from which to copy the calibration factors. If not, select **4** "Analysis set-up" and enter the desired set-up number.

* Select **2** to save the calibration factors.

* Enter the number of the new parameter set on line 4 and select **3** to copy the calibration factors.

The new parameter set now contains the same Calibration Factor and System Blank as the one copied.

DC-190 Operation Guide

DAILY START-UP

1 Gas @ 30 Psig.

2. Check that the acid bottle is 1/3 full.

3. Confirm that the IC chamber is 1/2 full **(gas off)**

4. Fill IC chamber by using the prime acid function.

5 Press $\boxed{\text{CARRIER}}$ Check that gas is flowing in IC chamber.

6. Ensure there is water in the dehumidifier.

7. Observe green lights on carrier & furnace.

8 Check for: flow rate 180-220cc/min, dehumidifier temp. 0-10°C, and furnace temp. 680°C. (Most applications)

9. Confirm or change Set-up number on display. **(Section 6.8)**

10. Check analysis and inlet mode.

11. Print Set-up.

12. If using the Boat, connect Teflon tubing to inlet part of dehumidifier. **(Fig. 4.15)**

13. If using ASM, clean the rinse bottle and fill it with acidified DI water. **(Few Drops of H_3PO_4)**

14. Observe for stable baseline **(Peak to Peak <.2mV)** before starting analysis.

DAILY SHUT-DOWN

1. Check that system is <u>not</u> in the **RUN** mode.

2. Push $\boxed{\text{CARRIER}}$ to turn off gas.

3. Leave furnace at operating temperature. **(Normally 680°C)**

4. Disconnect the Teflon tubing from the dehumidifier to boat at the boat inlet.

5 For total shut down turn OFF main power switch in the rear.

OPER. & CAL

1. Select analysis mode **(Table 6.1)**

2. Select inlet mode **(Table 6.2)**

3. Confirm or change volume. **(Fig. 6.1)**

4. For **CALIBRATION**, press $\boxed{\text{CAL}}$ to confirm or change concentration. **(Section 6.7)**

5. For manual injection, see **Section 6.3** for injection technique.

6. For ASM, confirm or change other parameters. **(Table 6.3)**

7. Refer to **Table 6.3** for ASM vial markers.

8. For RSM, see **Section 6.5**.

9. To complete **CALIBRATION**, see **Section 6.7**.

MAINTENANCE

Daily checks:
1. Printer paper
2. Gas supplies
3. IC chamber 1/2 full & acidified
4. Water in dehumidifier tube
5. Acid bottle 1/3 full
6. Gas flow 180-220cc/min
7. Temp. at set point
8. Dehumidifier temp 0-10°C

Weekly checks:
1. Daily checks plus
2. Replace septum in POC sparger every 40 injections.
3. Inspect TC inlet valve
4. Inspect combustion tube. Wipe inside area near top with wet Q-tips if necessary.
5. Inspect IC inlet valve
6. Clean IC reactor
7. Drain dehumidifier water & replace with acidified water. Flush several times if necessary.

Monthly checks:
1. Daily & weekly and/or:
2. Inspect & replace LiOH if necessary.
3. After ~160 hrs of operation, rinse catalyst, and combustion tube, replace silver wool (Section 7.1). Condition catalyst at 900°C for 1/2 hr with DI injections.
4. Inspect O-rings in TC inlet and bottom connector. Replace if necessary.

DO'S & DONT'S

1. **DO** Check the bottom connector when checking the combustion tube.

2. **DO** Use a Soap Film Bubble meter to check output gas flow rates.

3. **DO** leave furnace at 680°C except for long term shut down.

4. **DO** Condition new catalyst. 100ul of water every 5 min. for 2 hours at 900°C.

5. **DON'T** use Pyrex wool in the combustion tube.

6. **DO** clean combustion tube weekly if used heavily. DI injections @ 900°C for 1/2 hrs. Use good water–should stabilize at 1 to 3ppm or better.

7. **DO** check valve seal & O-rings monthly when inspecting TC & IC ports.

8. **DO** re-align TC & IC ports with ASM probe after inspections.

9. **DO** study flow diagram Figs 8-1 & 8.2.

10. **DO** acidify ASM rinse bottle

11. **DON'T** use ASM stirring time > 30 sec.

12. **DO** inject acidified water daily into TC port if non-acidified samples are analyzed. (3, 100ul inj. of pH1, HCl or HNO_3)

13. **DO** rinse (section 7.1) and condition catalyst (section 5.3) when catalyst is contaminated.

14. **DON'T** raise drain line higher than 1 1/2" above lab bench.

SECTION 10

STANDARDS PREPARATION AND SAMPLE HANDLING

10.1 STANDARDS PREPARATION

REAGENT WATER

<u>Use:</u>

Standards preparation, system blanks, sample dilution, cleaning, etc.

<u>Requirements:</u>

Deionized or distilled.
ASTM Type II reagent water or equivalent.

TOC level: Less than 0.2 mgC/L.

ACID SOLUTION

<u>Use:</u>

Automatic acid feed for IC chamber, sparge stations, autosampler.

<u>Requirements:</u>

Reagent water.
Phosphoric (H_3PO_4), sulfuric (H_2SO_4), or nitric (HNO_3) acid, concentrated, reagent grade.

<u>Do not use hydrochloric acid (HCl).</u>

<u>Preparation:</u>

Final volume: 100 ml.

<u>20% Phosphoric Acid Solution:</u>

Add 20 ml acid to 80 ml reagent water. Transfer to the acid bottle (4 oz borosilicate with open top screw cap).

If phosphoric acid is not available, 10% sulfuric acid or 5% nitric acid can be substituted.

Replace monthly.

TC and IC STOCK SOLUTIONS

Use:

Dilute to appropriate concentration for calibration or system check-out.

Requirements:

Reagent water.

Reagent-grade concentrated acid (H_3PO_4 or H_2SO_4) for TC stock only.

Standard compounds are reagent-grade, and must be dried to a constant weight. (See the table in the next page.)

Preparation:

Final volume: 100 mL.

Standard compound choice:
For system performance check and troubleshooting purposes, use a compound listed below. For routine analyses, use one of these, or any compound which might be more appropriate for your application.

Weigh the specified amount of the compound into a 100 ml volumetric flask. Add about 75 ml reagent water to dissolve the compound. Add about 0.1 ml acid to TC solutions to adjust pH below 3. Then fill to the mark.

Store stock solutions in amber borosilicate bottles with Teflon-lined closures at $4^{o}C$.

Replace monthly.

TC STOCK SOLUTIONS (Choose one):

Compound	Weight (g/100mL)	Concentration	Add Acid?
KHP ($C_8H_5KO_4$)	2.126	10,000 mgC/L	Yes
Sucrose ($C_{12}H_{22}O_{11}$)	2.375	10,000 mgC/L	Yes

IC STOCK SOLUTIONS (Choose one):

Compound	Weight (g/100mL)	Concentration	Add Acid?
Na_2CO_3 (Anhydrous)	0.883	1,000 mgC/L	No
$NaHCO_3$	0.699	1,000 mgC/L	No

Use this formula to determine the weight required to make 100 ml stock solutions using other compounds:

$$\text{g Compound} = \frac{\text{mw} \times \text{\%C}}{\text{N} \times 12.01}$$

where:

mw	=	molecular weight of compound
%C	=	concentration of standard in % carbon
N	=	number of carbon atoms per molecule
12.01	=	atomic weight of carbon

For example

For a 1% (10,000 mgC/L) solution of sucrose (mw = 342.29):

$$\frac{342.29 \times 1\%}{12 \times 12.01} = 2.375 \text{ g.}$$

TC and IC
WORKING STANDARDS

<u>Use:</u>

Calibration or system check-out.
Choose the standard concentration to match the working range of your samples.

<u>**Requirements:**</u>

Reagent water.
Clean volumetric flasks and volumetric pipets.

<u>**Preparation:**</u>

Final volume: Depends on concentrations.

Use larger volumes as concentration decreases. Make 1 liter volume at 10 mgC/L. Do not make final volume smaller than 100 ml.

TC solutions only: Maintain at pH 3 or lower.

Store standard solutions in amber borosilicate bottles with Teflon-lined closures at $4^{o}C$. **Minimize exposure to atmosphere.**

Bottle volume: Between 100 - 200 mL, depending upon the concentration.

Replace weekly.

<u>**System Performance Check:**</u> (Initial Start-Up)

Make 100 ml of 1000 mgC/L TC standard and 100 ml of 100 mgC/L IC standard.

POC STANDARD

Use:

Calibrate POC sparger.

Requirements:

Very clean 1 liter volumetric flask.

Reagent water.

Stir plate and Teflon coated stirbar.

Reagent grade compound.

Preparation:

Final volume: 1000 ml.

Compound Choice:

Benzene or chloroform is strongly recommended. Other compounds can be used if reliable results can be demonstrated. Use only benzene or chloroform for system performance check and troubleshooting.

WARNING!

BENZENE	DANGER! Extremely flammable. Suspected human carcinogen. Harmful if swallowed, inhaled or absorded through the skin. May affect the blood system.
CHLOROFORM	Warning! Suspected human carcinogen. Harmful if inhaled or swallowed. Skin and eye irritant and may produce toxic vapors if burned.

Please consult material safety data sheets for more precautions regarding these compounds.

Fill the 1 liter flask to the mark with reagent water. Add the stir bar and gently agitate water on stirplate for 1 - 2 minutes to degas. Inject a microliter quantity of the compound. Use the table or formula in the following page to determine the proper quantity to inject. The syringe needle should be well immersed in the water. Cap the flask and gently agitate the solution until it comes to equilibrium (approximately 5 minutes).

COMPOUND	VOLUME TO INJECT	CONCENTRATION
Benzene (C_6H_6)	12 ul	9.92 mgC/L
Chloroforom ($CHCl_3$)	67 ul	9.72 mgC/L

To make other concentrations or standards, use this formula:

$$\textbf{Concentration of POC Standard } C = \frac{V \times D \times F}{L}$$

where:

C = Concentration of standard (mgC/L)
V = Microliters of POC solvent injected
D = Density of POC solvent (mg/ul)
F = Fraction of carbon per molecule by weight
L = Volume in liters of water

10.2 SAMPLE HANDLING

Good laboratory practice is important in obtaining reliable analysis for carbon content of samples. Since carbon is everywhere in nature, it is very easy to contaminate a sample. Follow these guidelines for sample handling during collection, pretreatment, and analysis.

Syringe Handling:

Dedicate a syringe to a particular carbon range. When the syringe gets contaminated (indicated by sample or standard not completely wetting the inner barrel), draw chromic acid into the syringe a few times, then rinse well with reagent water.

Sample Bottles:

It is preferable to store and collect samples in glass containers. Plastic bottles should only be used if it is established that the specific type of container to be used does not contribute contaminating organics.

The sample collection bottles should be cleaned well before collecting the sample. The amount of cleaning necessary is dependent on the expected concentration of carbon in the sample. As a rule of thumb, the following levels are suggested:

*** Greater than 100 mgC/L**

- Wash bottle in hot, soapy water.
- Rinse with clean water.
- Plastic cap may be used, but try to use Teflon-lined cap.
- Analyze samples within 2 weeks.
- Treat standard bottles and sparge vials the same way.

*** Less than 100 mgC/L**

- Use amber bottle.
- Wash in hot, soapy water.
- Rinse with clean water.
- Swirl with chromic/sulfuric acid cleaning solution.
- Rinse with reagent water.
- Use Teflon-lined cap.
- Store sample at 4^{O}C.
- Analyze within two weeks.
- Treat standard bottles and sparge vials the same way.

Sample Pretreatment:

If a sample contains particulates larger than 0.5 mm or insoluble matter, homogenize with a blender or tissuemizer until the average particle size is less than 0.5 mm. Analyze these samples with the micropipettor or autosampler.

If the average particle size cannot be reduced to below 0.5 mm by homogenizing, dilute the sample with reagent water and blend again, or analyze the sample using the boat sampler.

* Below 100 mgC/L:

Minimize the sample handling and the blend time in order to minimize contamination and loss of volatiles. Analyze a blank with the same pretreatment as a sample.

APPENDIX A-8

Procedure for Chemical Oxygen Demand: Method 410.4

Chemical Oxygen Demand
EPA Method 410.4
with Hach DR/2000

1.0 Procedure

Perform analysis for Chemical Oxygen Demand on the Hach DR/2000 in accordance with manufacturer's instructions (Method 8000 attached).

2.0 Recordkeeping

Retain all worksheets, notes, and calculations of percent recovery on quality control samples as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform a method blank and one quality control sample made from a sucrose solution. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

4.0 References

"Chemical Oxygen Demand Method 410.4 (Colorimetric, Automated; Manual)", *Methods for Chemical Analysis of Water and Wastes - Revised March 1983*, (U. S.) Enviromental Protection Agencey, Cincinnati, OH, P84-128677

"5220 Chemical Oxygen Demand (COD)", "5220 D. Closed Reflux, Colorimetric Method", *Standard Methods for the Analysis of Water and Wastewater*, 19th ed., [American Public Health Association]

CHEMICAL OXYGEN DEMAND

Method 410.4 (Colorimetric, Automated; Manual)

STORET NO. 00340

1. Scope and Application
   1.1 This method covers the determination of COD in surface waters, domestic and industrial wastes.
   1.2 The applicable range of the automated method is 3–900 mg/l and the range of the manual method is 20 to 900 mg/l.
2. Summary of Method
   2.1 Sample, blanks and standards in sealed tubes are heated in an oven or block digestor in the presence of dichromate at 150°C. After two hours, the tubes are removed from the oven or digestor, cooled and measured spectrophotometrically at 600 nm.
3. Sample Handling and Preservation
   3.1 Collect the samples in glass bottles if possible. Use of plastic containers is permissible if it is known that no organic contaminants are present in the containers.
   3.2 Samples should be preserved with sulfuric acid to a pH < 2 and maintained at 4°C until analysis.
4. Interferences
   4.1 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion tubes to complex the chlorides.
5. Apparatus
   5.1 Drying oven or block digestor, 150°C
   5.2 Corning culture tubes, 16 x 100 mm or 25 x 150 mm with Teflon lined screw cap
   5.3 Spectrophotometer or Technicon AutoAnalyzer
   5.4 Muffle furnace, 500°C.
6. Reagents
   6.1 Digestion solution: Add 10.2 g $K_2Cr_2O_7$, 167 ml conc. H_2SO_4 and 33.3 g $HgSO_4$ to 500 ml of distilled water, cool and dilute to 1 liter.
   6.2 Catalyst solution: Add 22 g Ag_2SO_4 to a 4.09kg bottle of conc. H_2SO_4. Stir until dissolved.
   6.3 Sampler wash solution: Add 500 ml of conc H_2SO_4 to 500 ml of distilled water.
   6.4 Stock potassium acid phthalate: Dissolve 0.850 g in 800 ml of distilled water and dilute to 1 liter. 1 ml = 1 mg COD
      6.4.1 Prepare a series of standard solutions that cover the expected sample concentrations by diluting appropriate volumes of the stock standard.
7. Procedure
   7.1 Wash all culture tubes and screw caps with 20% H_2SO_4 before their first use to prevent contamination. Trace contamination may be removed from the tubes by igniting them in a muffle oven at 500°C for 1 hour.

Pending approval for Section 304(h), CWA
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7.2 Automated

7.2.1 Add 2.5 ml of sample to the 16 x 100 mm tubes.

7.2.2 Add 1.5 ml of digestion solution (6.1) and mix.

7.2.3 Add 3.5 ml of catalyst solution (6.2) carefully down the side of the culture tube.

7.2.4 Cap tightly and shake to mix layers.

7.2.5 Process standards and blanks exactly as the samples.

7.2.6 Place in oven or block digestor at 150°C for two hours.

7.2.7 Cool, and place standards in sampler in order of decreasing concentration. Complete filling sampler tray with unknown samples.

7.2.8 Measure color intensity on AutoAnalyzer at 600 nm.

7.3 Manual

7.3.1 The following procedure may be used if a larger sample is desired or a spectrophotometer is used in place of an AutoAnalyzer.

7.3.2 Add 10 ml of sample to 25 x 150 mm culture tube.

7.3.3 Add 6 ml of digestion solution (6.1) and mix.

7.3.4 Add 14 ml of catalyst solution (6.2) down the side of culture tube.

7.3.5 Cap tightly and shake to mix layers.

7.3.6 Place in oven or block digestor at 150°C for 2 hours.

7.3.7 Cool, allow any precipitate to settle and measure intensity in spectrophotometer at 600 nm. Use only optically matched culture tubes or a single cell for spectrophotometric measurement.

8. Calculation

8.1 Prepare a standard curve by plotting peak height or percent transmittance against known concentrations of standards.

8.2 Compute concentration of samples by comparing sample response to standard curve.

9. Precision and Accuracy

9.1 Precision and accuracy data are not available at this time.

Bibliography

1. Jirka, A. M., and M. J. Carter, "Micro-Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand." Anal. Chem. 47:1397. (1975).

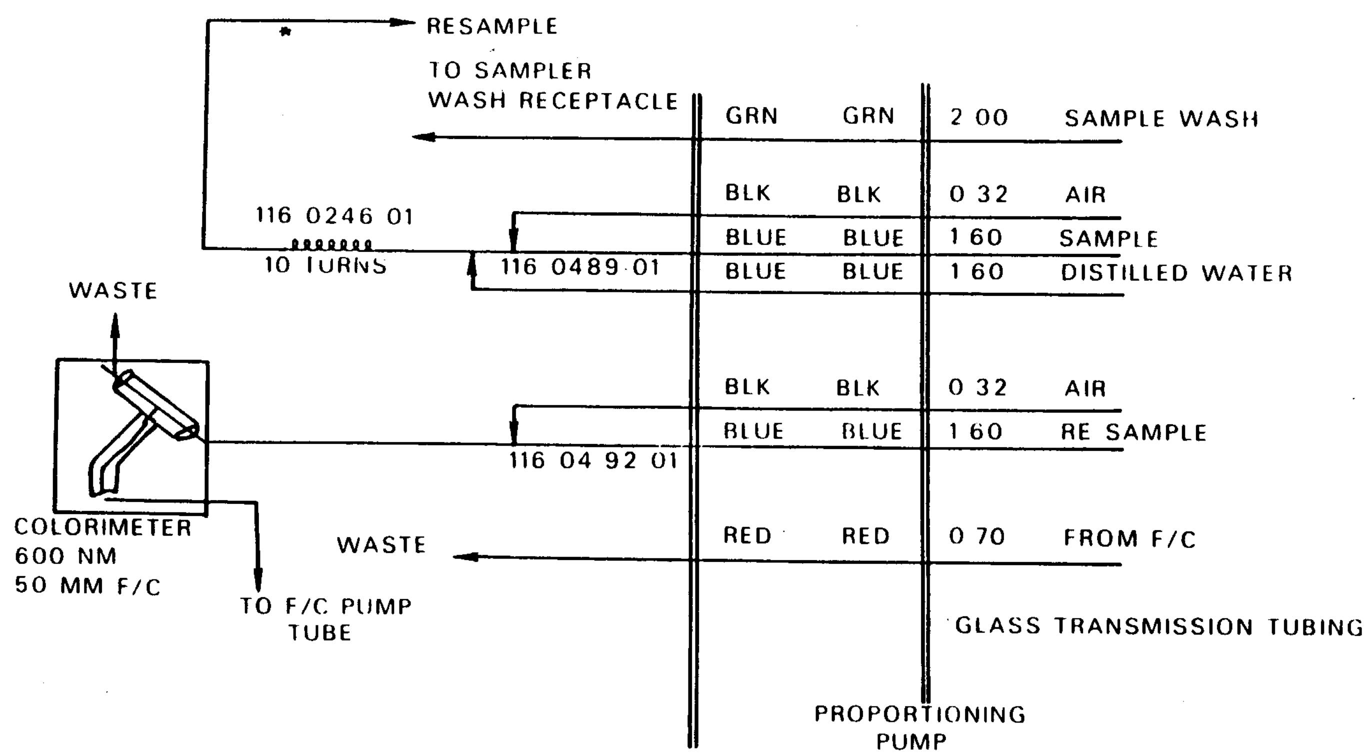


FIGURE 1 C O D MANIFOLD AA1 OR AA 11

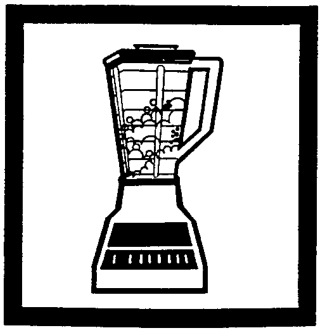
Method 8000

OXYGEN DEMAND, CHEMICAL

For water, wastewater and seawater

Reactor Digestion Method*; USEPA approved for reporting**

DIGESTION



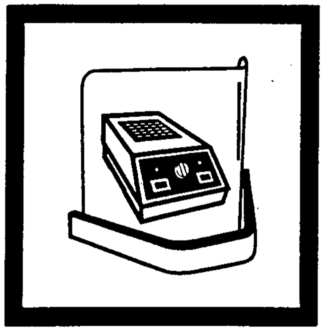
1. Homogenize 500 mL of sample for 2 minutes in a blender.

0 to 15,000 mg/L Note: *Homogenize 100 mL of sample. Pour the homogenized sample into a 250–mL beaker and stir with a magnetic stirrer.*

Note: *Blending ensures distribution of solids and improves accuracy and reproducibility.*

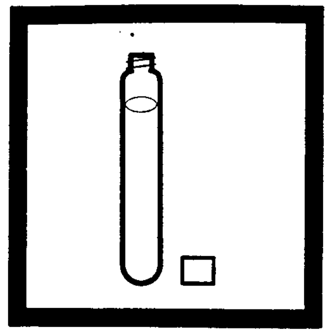
Note: *If samples cannot be analyzed immediately, see Sampling and Storage following these procedures.*

Caution: *Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidently misused. Please read all warnings and the safety section of this manual. Appropriate eye protection and clothing should be used for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.*



2. Turn on the COD Reactor. Preheat to 150 °C. Place the plastic shield in front of the reactor.

Caution: *Ensure safety devices are in place to protect analyst from splattering should reagent leaking occur.*

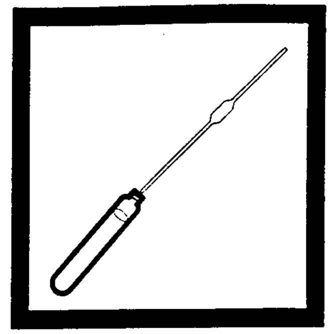


3. Remove the cap of a COD Digestion Reagent Vial for the appropriate range:

Sample Concentration Range (mg/L)	COD Digestion Reagent Vial Type
0 to 150	Low Range
0 to 1,500	High Range
0 to 15,000	High Range Plus

Use the cap tool provided to loosen the High Range Plus vials caps.

Note: *The reagent mixture is light–sensitive. Keep unused vials in the opaque shipping container, in a refrigerator if possible. The amount of light striking the vials during the test will not affect results.*



4. Hold the vial at a 45–degree angle. Pipet 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) of sample into the vial.

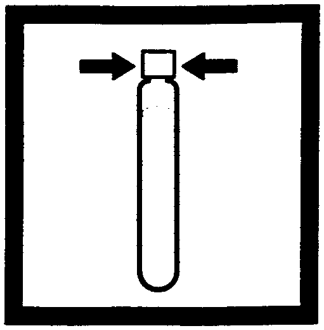
0 to 15,000 mg/L Note: *Pipet only 0.20 mL of sample, not 2.00 mL, using a TenSette Pipet. For greater accuracy a minimum of three replicates should be analyzed and the results averaged.*

Note: *Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Do not run tests with vials which have been spilled. If contact occurs, wash with running water.*

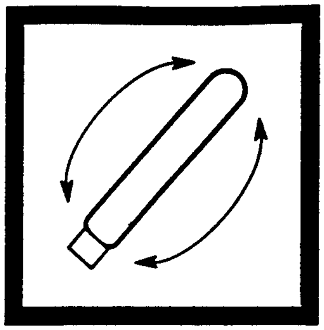
Note: *For proof of accuracy, use COD standard solutions (preparation given in the Accuracy Check) in place of the sample.*

*Jirka, A.M.; Carter, M.J. *Analytical Chemistry*, 1975, 47(8), 1397

**Federal Register*, April 21, 1980, 45(78), 26811–26812

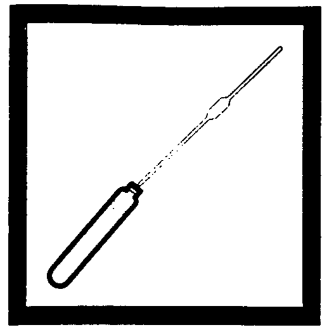


5. Replace the vial cap tightly. Use the cap tool provided, if necessary. Rinse the COD vial with deionized water and wipe the vial clean with a paper towel.



6. Hold the vial by the cap and over a sink. Invert gently several times to mix the contents. Place the vial in the preheated COD Reactor.

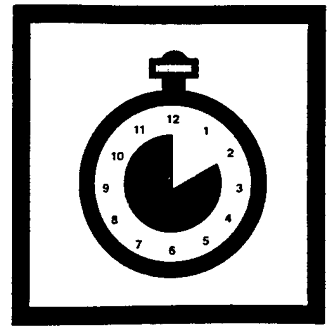
Note: *The vial will become very hot during mixing.*



7. Prepare a blank by repeating Steps 3 to 6, substituting 2.00 mL (0.2 mL for the 0 to 15,000 mg/L range) deionized water for the sample.

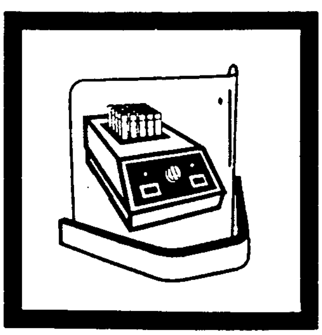
Note: *Be sure the pipet is well rinsed, or use a clean pipet.*

Note: *One blank must be run with each set of samples. All tests (samples and blank) should be run with the same lot of vials. The lot number appears on the container label.*

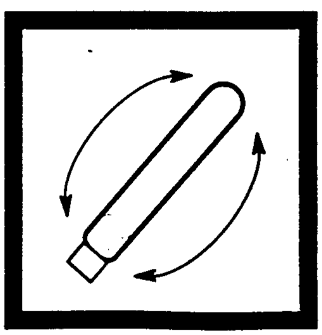


8. Heat the vials for 2 hours.

Note: *Many wastewater samples containing easily oxidized materials are digested completely in less than two hours. If desired, measure the concentration (while still hot) at 15 minute intervals until it remains unchanged. At this point, the sample is completely digested. Cool the vials to room temperature for final measurement.*



9. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120 °C or less.



10. Invert each vial several times while still warm. Place the vials into a rack. Wait until the vials have cooled to room temperature.

Note: *If a pure green color appears in the reacted sample, the reagent capacity may have been exceeded. Measure the COD and, if necessary, repeat the test with a diluted sample.*

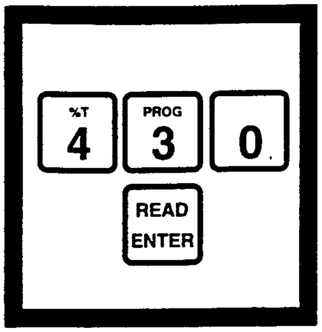
Colorimetric
or
Titrimetric

11. Use one of the following analytical techniques to determine the sample concentration:

Colorimetric determination, 0 to 150 mg/L COD
Colorimetric determination, 0 to 1,500 mg/L COD
Colorimetric determination, 0 to 15,000 mg/L COD
Buret titration

 COD CH, etc 0-150

COLORIMETRIC DETERMINATION, 0 to 150 mg/L COD



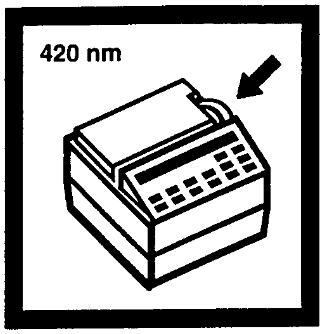
1. Enter the stored program number for chemical oxygen demand (COD), low range.

Press: **4 3 0 READ/ENTER**

The display will show:
DIAL nm TO 420

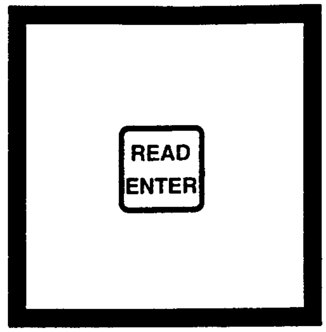
Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.



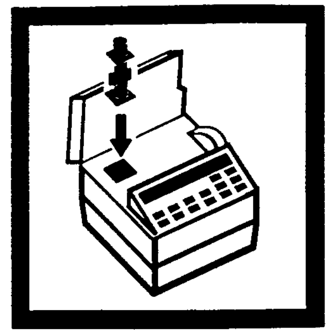
2. Rotate the wavelength dial until the small display shows:
420 nm

Note: Approach the wavelength setting from the higher to lower values.

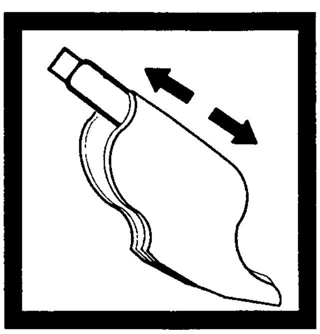


3. Press: **READ/ENTER**

The display will show:
mg/l COD L

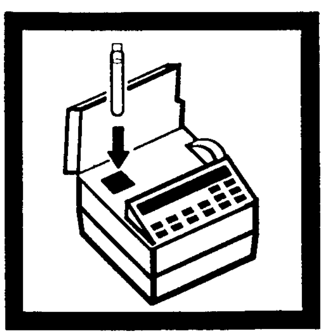


4. Place the COD Vial Adapter into the cell holder with the marker to the right.



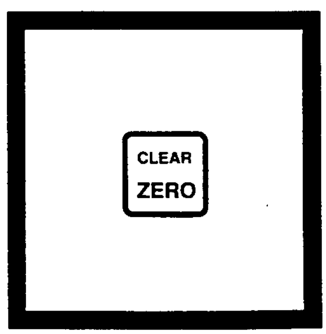
5. Clean the outside of the blank with a towel.

Note: Wiping with a damp towel, followed by a dry one will remove fingerprints or other marks.



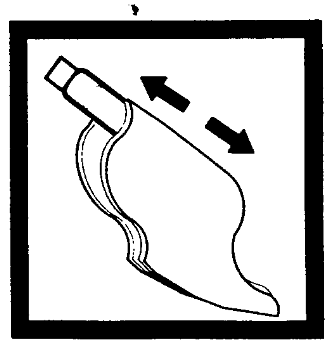
6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: The blank is stable when stored in the dark; see Blanks for Colorimetric Determination following these procedures.

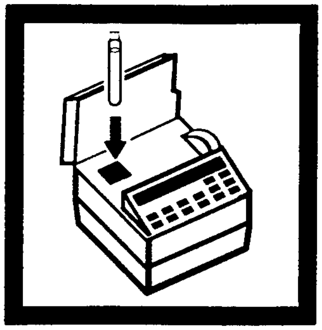


7. Press: **ZERO**

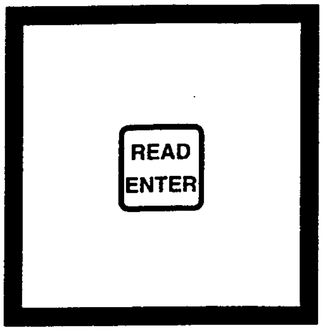
The display will show:
WAIT
then:
0. mg/l COD L



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.



10. Press: **READ/ENTER**

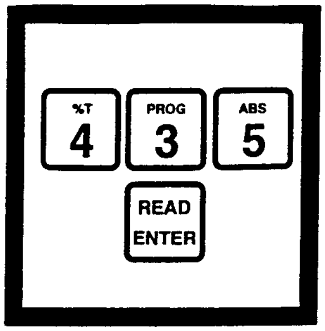
The display will show:
WAIT
then the result in mg/L COD will be displayed.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: For most accurate results with samples near 150 mg/L COD, repeat the analysis with a diluted sample.

COD
CH ETC. 0-15K

COLORIMETRIC DETERMINATION, 0 to 1,500 and 0 to 15,000 mg/L COD



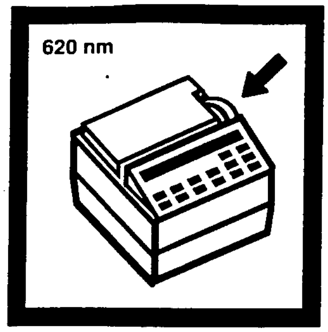
1. Enter the stored program number for chemical oxygen demand, high range.

Press: **4 3 5 READ/ENTER**

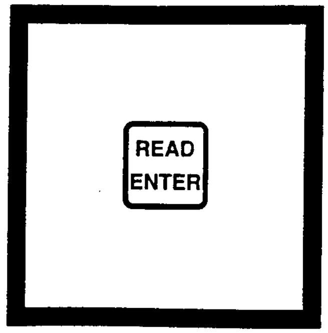
The display will show:
DIAL nm TO 620

Note: DR/2000s with software versions 3.0 and greater will display "P" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

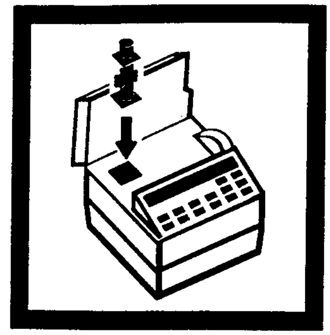


2. Rotate the wavelength dial until the small display shows:
620 nm

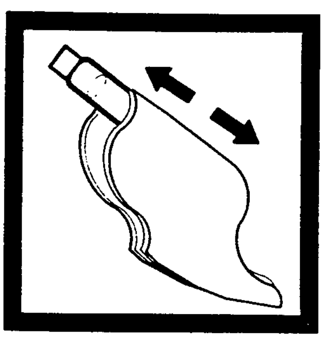


3. Press: **READ/ENTER**

The display will show:
mg/l COD H

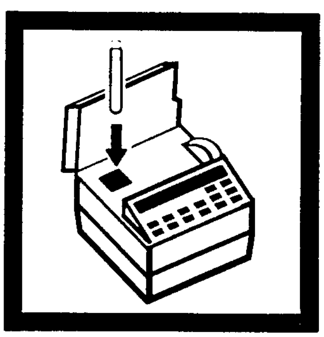


4. Place the COD Vial Adapter into the cell holder with the marker to the right.



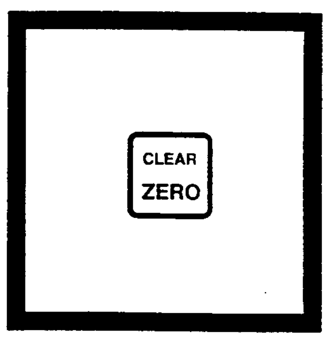
5. Clean the outside of the blank with a towel.

Note: Wiping with a damp towel followed by a dry one will remove fingerprints or other marks.



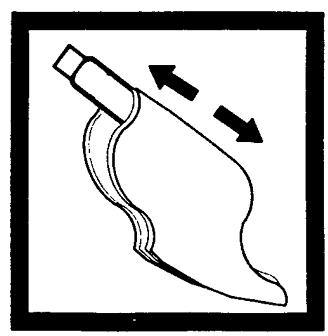
6. Place the blank into the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.

Note: The blank is stable when stored in the dark. See Blanks for Colorimetric Determination following these procedures.

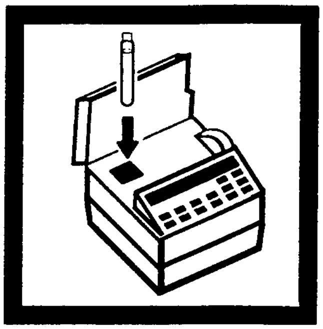


7. Press: **ZERO**

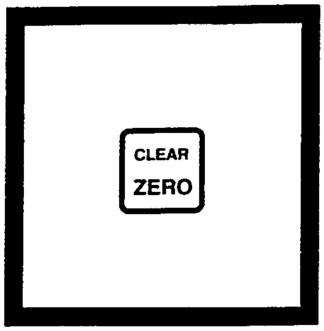
The display will show:
WAIT
then:
0. mg/l COD H



8. Clean the outside of the sample vial with a towel.



9. Place the sample vial in the adapter with the Hach logo facing the front of the instrument. Place the cover on the adapter.



10. Press: **READ/ENTER**

The display will show:
WAIT
then the result in mg/L COD will be displayed.

0 to 15,000 mg/L Note: *When High Range Plus COD Digestion Reagent Vials are used, multiply the displayed value by ten.*

Note: *In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

Note: *For most accurate results with samples near 1,500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.*

APPENDIX A-9

Procedure for Ammonia Nitrogen: Method 350.1

1.0 PURPOSE

This procedure provides a method for the determination of ammonia in drinking and surface waters.

2.0 SCOPE

2.1 This method covers the determination of ammonia in drinking and surface waters.

2.2 The method is based on reactions that are specific for the ammonium ion.

2.3 The applicable range is 0.1 to 20.0 mg N/L as NH3.

3.0 SUMMARY

This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, then with sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Ammonia, Method 350.1 (Colorimetric, Automated Phenate)."

4.2 U.S. Environmental Protection Agency, 40 CFR Part 36 Table 1B, footnote 6, 1994.

4.3 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-06-1-A, "Determination Of Ammonia By Flow Injection Analysis, Colorimetry."

4.4 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the Analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter (preferred - 1 mL of 1N H_2SO_4 per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.

6.2.2.2 Color, turbidity and certain organic species may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis. Sample color may be corrected for by running the samples through the

manifold without color formation (omit Sodium Phenolate, reagent 1). The ammonium concentration is determined by subtracting the value obtained without color formation from the value obtained with color formation.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimetric detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Heating Unit

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

6.4 Reagents and Standards

6.4.1 Preparation of Reagents -

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas all solutions except the standards, Sodium Phenolate (Reagent 1) and Sodium Hypochlorite (Reagent 2) with helium. Bubble helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. Sodium Phenolate**

CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed in the body through the skin.

By Volume: In a **1 L** volumetric flask, dissolve **88 mL** of **88% liquefied phenol** or **83 g crystaline phenol** (C_6H_5OH) in approximately **600 mL water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and mix. Do not degas this reagent.

By weight: To a tared **1 L** container, add **888 g water**. Add **94.2 g** of **88 liquefied phenol** or **83 g crystalline phenol** (C_6H_5OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and invert to mix. Do not degas this reagent.

6.4.1.2 **Reagent 2. Sodium Hypochlorite**

By Volume: In a **500 mL** volumetric flask, dilute **250 mL Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] to mark with **water**. Invert to mix.

By weight: To a tared **500 mL** container, add **250 g Regular Clorox bleach** [5.25% sodium hypochlorite (NaOCl), The Clorox Company, Oakland, CA] and **250 g water**. Invert to mix.

6.4.1.3 **Reagent 3. Buffer**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetate dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **5.5 g sodium hydroxide** (NaOH) in about **900 mL water**. Dilute to the mark and invert or stir to mix.

By weight: To a tared **1 L** container, add **50.0 g disodium ethylenediamine tetraacetate dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **5.5 g sodium hydroxide** (NaOH). Add **968 g water**. Invert or stir to mix.

6.4.1.4 **Reagent 4. Sodium Nitroprusside**

By Volume: In a **1 L** volumetric flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferrricyanide [$Na_2Fe(CN)5NO \cdot 2H2O$]) dilute to the mark with **water**. Stir or shake to mix.

By weight: To a tared **1 L** flask, dissolve **3.50 g sodium nitroprusside** (Sodium Nitroferrricyanide [$Na_2Fe(CN)_5NO \cdot 2H_2O$]) and **1000 g water**. Stir or shake to mix.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneously for PO_4-P, NH_3-N and NO_2-N + NO_3-N. Also included is the preparation of a NO_2-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO_4**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) for one hour at 105°C. In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH_3**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO_3^-**

In a **1 L** volumetric flask dissolve **7.220 g potassium nitrate** (KNO_3) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO_2^-**

In a **1 L** volumetric flask dissolve **4.93 g sodium nitrate** ($NaNO_2$) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO_4-P, NH_3-N and NO_3-N**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with **water** and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO_2^-**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **20 mL Stock Nitrate Standard** (standard 4). Dilute to mark with **water** and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO_4^{3-}, 31.06 mg N/L as NH_4, and 27.11 mg N/L as NO_3^-.**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the E M Science **1000 mg/L Phosphate Standard Solution** (326.1 mg P/L), **20 mL** of the E M Science **1000 mg/L Ammonia Standard Solution** (776.5 mg N/L), and **60 mL** of the E M Science **1000 mg/L Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water**.

	Calibration Standards Concentration mg/L	Prepared From Concentration mg/L	Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H_2SO_4 added per 100 mL, add **5 mL** of **1N H_2SO_4** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO_2^-**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water,** add **5 mL** of **1N H_2SO_4** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO_4, 1.55 mg N/L as NH_3, and 1.36 mg N/L as NO_3^-.**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water,** add **10 mL** of 1N H_2SO_4 and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above three QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.2.

7.1.4 Enter data system parameters as in section 9.1.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Allow 15 minutes for heating unit to warm up to 60°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 1).

7.1.10 At end of run, remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 To prevent baseline drifts, peaks that are too wide, or other problems with NH3-N precision, clean the NH_3-N manifold by placing the manifold reagent lines in 1M hydrochloric acid (1 volume concentrated HCl added to 11 volumes of water). Pump for about 5 minutes.

7.1.12 Remove all reagent lines from the hydrochloric acid and place them in water. Pump until the HCl is thoroughly washed out (about 5 minutes).

7.1.13 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual*, page 43, "Task 11 - Creating a Custom Report")

7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.

7.2.5 Report results in mg NH_3-N/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters

Method Filename:	PANHANOW.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name:	Ammonia (NH_3)-N
Concentration Units:	mg NH_3-N/L
Chemistry:	Direct
Inject to Peak Start (s):	28.0
Peak Base Width (s):	21.000
% Width Tolerance:	100.000
Threshold:	8000.000
Autodilution Trigger:	Off
QuickChem Method:	10-107-06-1-A

Calibration Data:

Levels: (mg NH_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		

Sampler Timing:

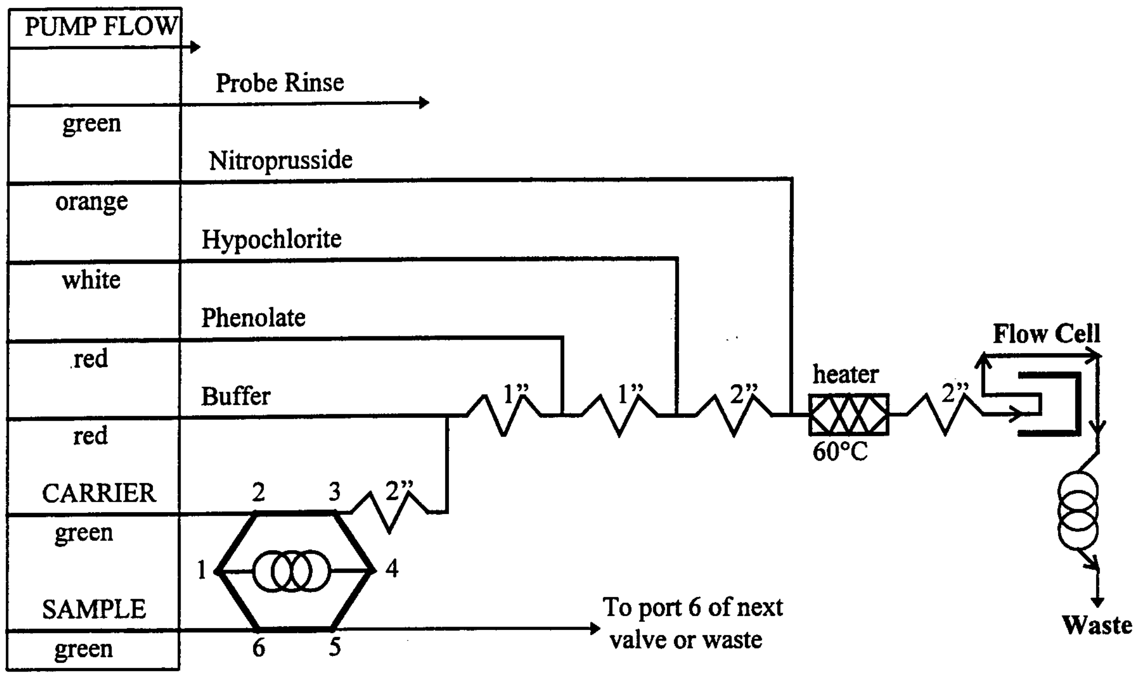
Method Cycle Period:	70.0
Min. Probe in Wash Period:	9.0
Probe in Sample Period:	30.0

Valve Timing:

Method Cycle Period:	70.0
Sample Reaches 1st Valve:	18.0
Valve:	On
Load Time:	0.0
Load period	25.0
Inject Period:	45.0

Sample Loop: 13 cm x 0.5 mm i.d.

9.2 Ammonia Manifold Diagram



Sample Loop = 13 cm x 0.5 mm i.d.

Interference Filter = 630 nm

Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**. The sample loop uses **0.5 mm (0.022”) i.d.** tubing.

1 is **70 cm** of tubing on a **4.5 cm** coil support.

Apparatus: The  includes 650 cm of tubing wrapped around the heater
60°C
block at the specified temperature.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

NITROGEN, AMMONIA

Method 350.1 (Colorimetric, Automated Phenate)

STORET NO. Total 00610
Dissolved 00608

1. Scope and Application
   1.1 This method covers the determination of ammonia in drinking, surface, and saline waters, domestic and industrial wastes in the range of 0.01 to 2.0 mg/1 NH_3 as N. This range is for photometric measurements made at 630–660 nm in a 15 mm or 50 mm tubular flow cell. Higher concentrations can be determined by sample dilution. Approximately 20 to 60 samples per hour can be analyzed.
2. Summary of Method
   2.1 Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside.
3. Sample Handling and Preservation
   3.1 Preservation by addition of 2 ml conc. H_2SO_4 per liter and refrigeration at 4°C.
4. Interferences
   4.1 Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems during analysis. A 5% EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For sea water a sodium potassium tartrate solution is used.
   4.2 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.
5. Apparatus
   5.1 Technicon AutoAnalyzer Unit (AAI or AAII) consisting of:
      5.1.1 Sampler.
      5.1.2 Manifold (AAI) or Analytical Cartridge (AAII).
      5.1.3 Proportioning pump.
      5.1.4 Heating bath with double delay coil (AAI).
      5.1.5 Colorimeter equipped with 15 mm tubular flow cell and 630–660 nm filters.
      5.1.6 Recorder.
      5.1.7 Digital printer for AAII (optional).

Approved for NPDES following preliminary distillation, Method 350.2.
Issued 1974
Editorial revision 1978

6. Reagents

6.1 Distilled water: Special precaution must be taken to insure that distilled water is free of ammonia. Such water is prepared by passage of distilled water through an ion exchange column comprised of a mixture of both strongly acidic cation and strongly basic anion exchange resins. The regeneration of the ion exchange column should be carried out according to the instruction of the manufacturer.

NOTE 1: All solutions must be made using ammonia-free water.

6.2 Sulfuric acid 5N: Air scrubber solution. Carefully add 139 ml of conc. sulfuric acid to approximately 500 ml of ammonia-free distilled water. Cool to room temperature and dilute to 1 liter with ammonia-free distilled water.

6.3 Sodium phenolate: Using a 1 liter Erlenmeyer flask, dissolve 83 g phenol in 500 ml of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 liter with distilled water.

6.4 Sodium hypochlorite solution: Dilute 250 ml of a bleach solution containing 5.25% NaOCl (such as "Clorox") to 500 ml with distilled water. Available chlorine level should approximate 2 to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

6.5 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) and approximately six pellets of NaOH in 1 liter of distilled water.

NOTE 2: On salt water samples where EDTA solution does not prevent precipitation of cations, sodium potassium tartrate solution may be used to advantage. It is prepared as follows:

6.5.1 Sodium potassium tartrate solution: 10% $NaKC_4H_4O_6 \cdot 4H_2O$. To 900 ml of distilled water add 100 g sodium potassium tartrate. Add 2 pellets of NaOH and a few boiling chips, boil gently for 45 minutes. Cover, cool, and dilute to 1 liter with ammonia-free distilled water. Adjust pH to 5.2 ±.05 with H_2SO_4. After allowing to settle overnight in a cool place, filter to remove precipitate. Then add 1/2 ml Brij-35[4] (available from Technicon Corporation) solution and store in stoppered bottle.

6.6 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside in 1 liter of distilled water.

6.7 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH_4Cl, dried at 105°C, in distilled water, and dilute to 1000 ml. 1.0 ml = 1.0 mg NH_3–N.

6.8 Standard Solution A: Dilute 10.0 ml of stock solution (6.7) to 1000 ml with distilled water. 1.0 ml = 0.01 mg NH_3–N.

6.9 Standard solution B: Dilute 10.0 ml of standard solution A (6.8) to 100.0 ml with distilled water. 1.0 ml = 0.001 mg NH_3–N.

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6.10 Using standard solutions A and B, prepare the following standards in 100 ml volumetric flasks (prepare fresh daily):

NH_3–N; mg/l	ml Standard Solution/100 ml
	Solution B
0.01	1.0
0.02	2.0
0.05	5.0
0.10	10.0
	Solution A
0.20	2.0
0.50	5.0
0.80	8.0
1.00	10.0
1.50	15.0
2.00	20.0

NOTE 3: When saline water samples are ar¬lyzed, Substitute Ocean Water (SOW) should be used for preparing the above standards used for the calibration curve; otherwise, distilled water is used. If SOW is used, subtract its blank background response from the standards before preparing the standard curve.

Substitute Ocean Water (SOW)

NaCl	24.53 g/l	$NaHCO_3$	0.20 g/l
$MgCl_2$	5.20 g/l	KBr	0.10 g/l
Na_2SO_4	4.09 g/l	H_3BO_3	0.03 g/l
$CaCl_2$	1.16 g/l	$SrCl_2$	0.03 g/l
KCl	0.70 g/l	NaF	0.003 g/l

7. Procedure

7.1 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples. For example, if the samples have been preserved with 2 ml conc. H_2SO_4/liter, the wash water and standards should also contain 2 ml conc. H_2SO_4/liter.

7.2 For a working range of 0.01 to 2.00 mg NH_3–N/1 (AAI), set up the manifold as shown in Figure 1. For a working range of .01 to 1.0 mg NH_3–N/1 (AAII), set up the manifold as shown in Figure 2. Higher concentrations may be accommodated by sample dilution.

7.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a stable baseline with all reagents, feeding distilled water through sample line.

7.4 For the AAI system, sample at rate of 20/hr, 1:1. For the AAII use a 60/hr 6:1 cam with a common wash.

7.5 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

7.6 Switch sample line from distilled water to sampler and begin analysis.

8. Calculations

8.1 Prepare appropriate standard curve derived from processing ammonia standards through manifold. Compute concentration of samples by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3–N/1, the standard deviation was ±0.005.

9.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3–N/1, recoveries were 107% and 99%, respectively.

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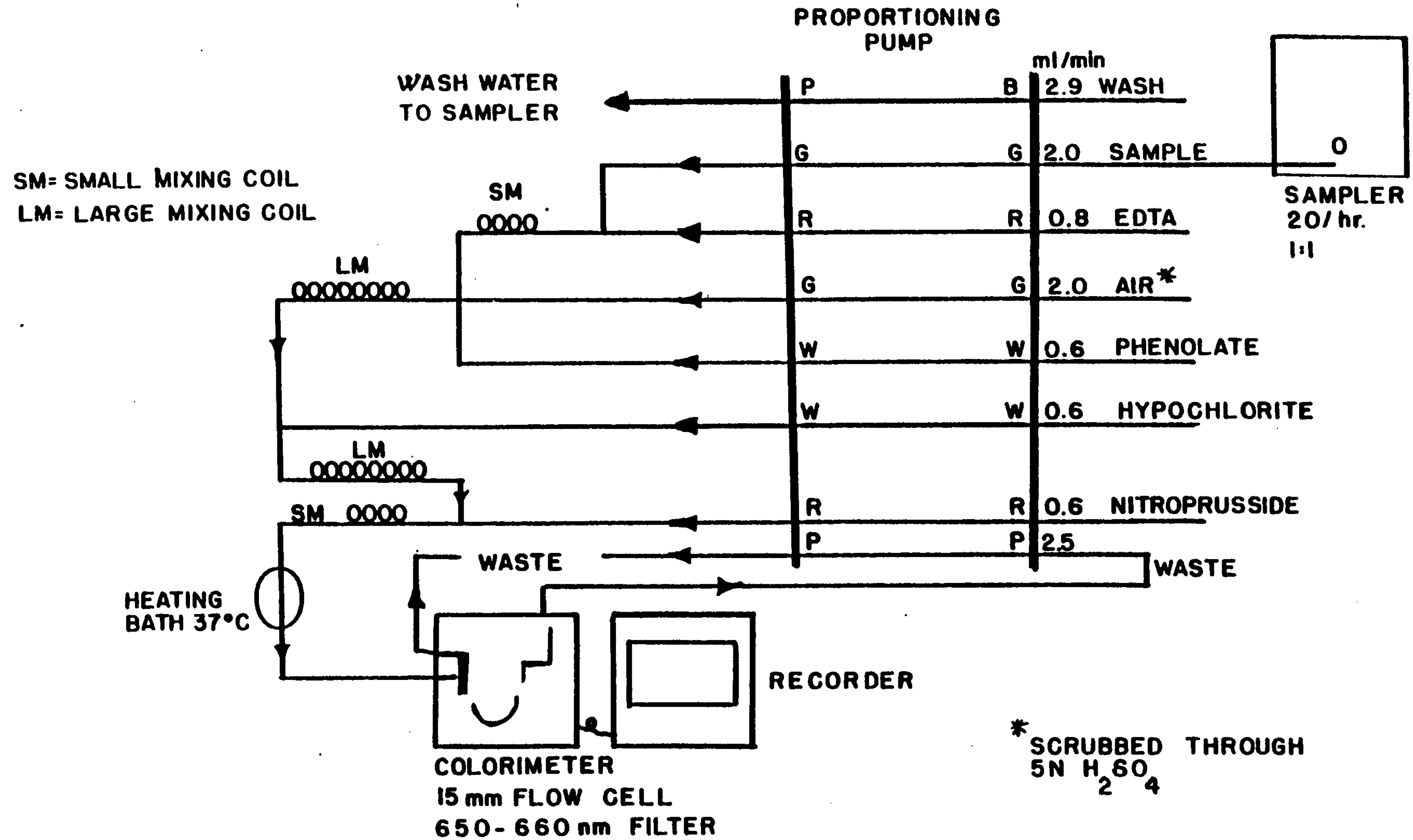

FIGURE 1 AMMONIA MANIFOLD AA I

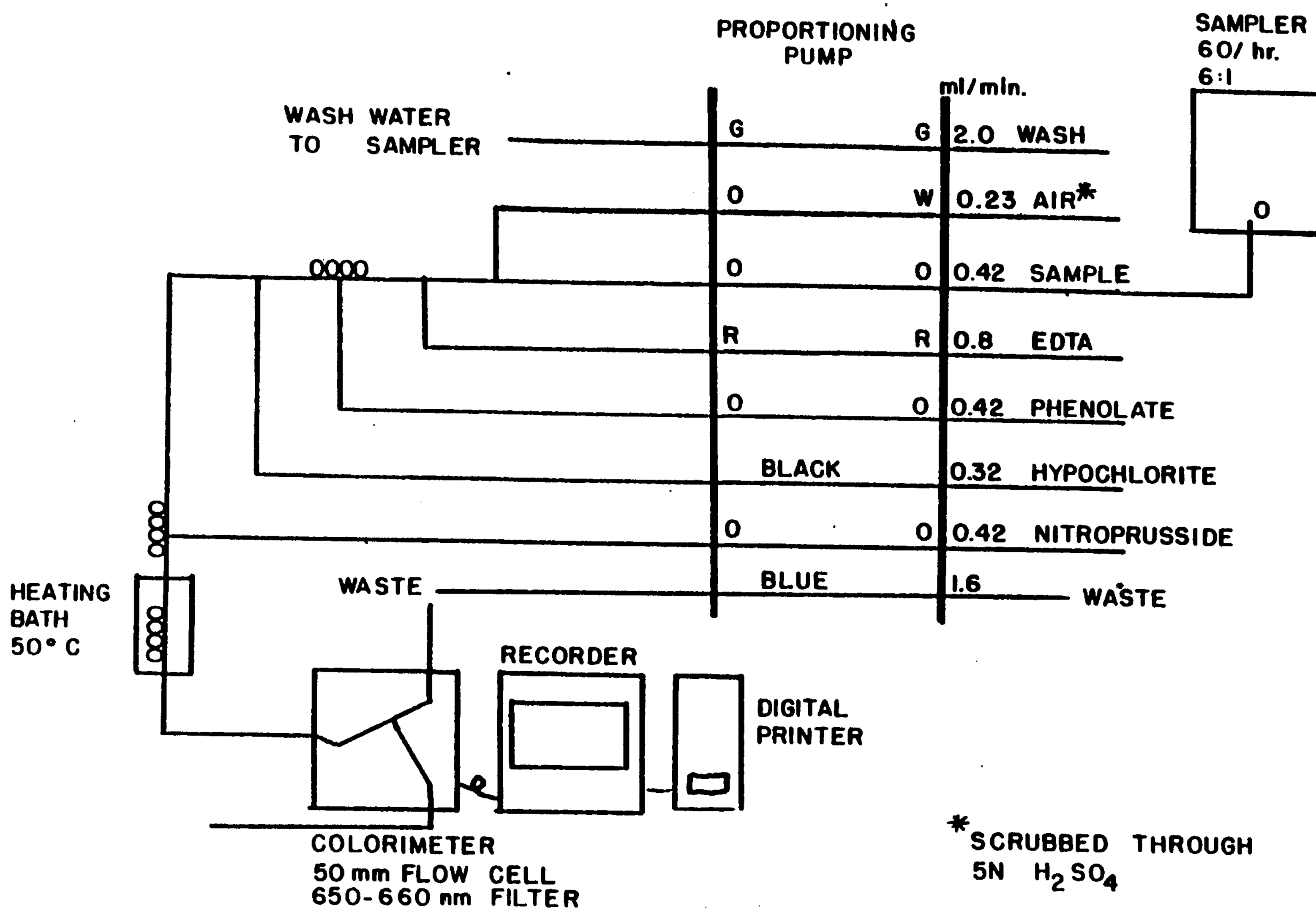

FIGURE 2. AMMONIA MANIFOLD AA II

APPENDIX A-10

Procedure for Total Kjeldahl Nitrogen: Method 351 Series

Table of Contents – Appendix A-10

Documents
QC Document – Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)
LACHAT Instruments – Quik Chem Method 10-107-06-2-D
LACHAT Instruments – Quik Chem Method 10-107-06-2-E
Technicon Auto Analyzer II – Industrial Method No. 334-74W/B

Nitrogen, Total Kjehldahl - Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

1.0 Procedure

Perform analysis for Total Kjehldahl Nitrogen (Method 351.2) in accordance with procedures for the Technicon II AutoAnalyzer, or for the Lachat Quick Chem 8000 flow injection analyzer as attached.

2.0 Recordkeeping

Retain all machine printouts, worksheets, percent recovery calculations of quality control samples, and notes.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or sample duplicate.

NITROGEN, KJELDAHL, TOTAL

Method 351.2 (Colorimetric, Semi-Automated Block Digester, AAII)

STORET NO. 00625

1. Scope and Application
   1.1 This method covers the determination of total Kjeldahl nitrogen in drinking and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogeneous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines. The applicable range of this method is 0.1 to 20 mg/1 TKN. The range may be extended with sample dilution.
2. Summary of Method
   2.1 The sample is heated in the presence of sulfuric acid, K_2SO_4 and $HgSO_4$ for two and one half hours. The residue is cooled, diluted to 25 ml and placed on the AutoAnalyzer for ammonia determination. This digested sample may also be used for phosphorus determination.
3. Definitions
   3.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.
   3.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free-ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value.
4. Sample Handling and Preservation
   4.1 Samples may be preserved by addition of 2 ml of conc H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic nitrogen to ammonia may occur. Therefore, samples should be analyzed as soon as possible.
5. Apparatus
   5.1 Block Digestor—40
   5.2 Technicon Manifold for Ammonia (Figure 1)
   5.3 Chemware TFE (Teflon boiling stones), Markson Science, Inc., Box 767, Delmar, CA 92014)
6. Reagents
   6.1 Mercuric Sulfate: Dissolve 8 g red mercuric oxide (HgO) in 50 ml of 1:4 sulfuric acid (10 ml conc H_2SO_4: 40 ml distilled water) and dilute to 100 ml with distilled water.
   6.2 Digestion Solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 in 700 ml of distilled water and 200 ml of conc H_2SO_4. Add 25 ml of mercuric sulfate solution and dilute to 1 liter.

Pending approval for NPDES
Issued 1978

6.3 Sulfuric Acid Solution (4%): Add 40 ml of conc. sulfuric acid to 800 ml of ammonia free distilled water, cool and dilute to 1 liter.

6.4 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide in 900 ml of ammonia-free distilled water and dilute to 1 liter.

6.5 Stock Sodium Potassium Tartrate Solution (20%): Dissolve 200 g sodium potassium tartrate in about 800 ml of ammonia-free distilled water and dilute to 1 liter.

6.6 Stock Buffer Solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) in about 800 ml of ammonia free water. Add 20 g of sodium hydroxide and dilute to 1 liter.

6.7 Working Buffer Solution: Combine the reagents in the stated order; add 250 ml of stock sodium potassium tartrate solution (6.5) to 200 ml of stock buffer solution (6.6) and mix. Add xx ml sodium hydroxide solution (6.4) and dilute to 1 liter. See concentration ranges, Table I, for composition of working buffer.

6.8 Sodium Salicylate/Sodium Nitroprusside Solution: Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in about 600 ml of ammonia free water and dilute to 1 liter.

6.9 Sodium Hypochlorite Solution: Dilute 6.0 ml sodium hypochlorite solution (clorox) to 100 ml with ammonia free distilled water.

6.10 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl in distilled water and bring to volume in a 1 liter volumetric flask. 1 ml = 1.0 mg NH_3-N.

7. Procedure

Digestion

7.1 To 20 or 25 ml of sample, add 5 ml of digestion solution (6.2) and mix (use a vortex mixer).

7.2 Add (4–8) Teflon boiling stones (5.3). Too many boiling chips will cause the sample to boil over.

7.3 With Block Digestor in manual mode set low and high temperature at 160°C and preheat unit to 160°C. Place tubes in digestor and switch to automatic mode. Set low temperature timer for 1 hour. Reset high temperature to 380°C and set timer for 2 1/2 hours.

7.4 Cool sample and dilute to 25 ml with ammonia free water.

Colorimetric Analysis

7.5 Check the level of all reagent containers to ensure an adequate supply.

7.6 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the Sampler IV and start the proportioning pump.

7.7 Flush the Sampler IV wash receptacle with about 25 ml of 4.0% sulfuric acid (6.3).

7.8 When reagents have been pumping for at least five minutes, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

TABLE 1

CONCENTRATION RANGES
(NITROGEN)

	Dilution loops						
	Initial sample		Resample		Approx.	Range	ml stock NaOH per liter
No.	Sample line	Diluent line	Resample line	Diluent line	std. cal. setting	PPM N (±10%)	working buffer solution
1	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–0.5	250
2	.80 (RED/RED)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–1.5	250
3	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	700	0–1	120
4	.16 (ORN/YEL)	.80 (RED/RED)	.32 (BLK/BLK)	.80 (RED/RED)	100	0–5	120
5	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	700	0–2	80
6	.16 (ORN/YEL)	.80 (RED/RED)	.16 (ORN/YEL)	.80 (RED/RED)	100	0–10	80

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7.9 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

7.10 After a stable baseline has been obtained start the Sampler.

8. Calculations

8.1 Prepare standard curve by plotting peak heights of processed standards against concentration values. Compute concentrations by comparing sample peak heights with standard curve.

9. Precision and Accuracy

9.1 In a single laboratory (EMSL), using sewage samples of concentrations of 1.2, 2.6, and 1.7 mg N/1, the precision was ±0.07, ±0.03 and ±0.15, respectively.

9.2 In a single laboratory (EMSL), using sewage samples of concentrations of 4.7 and 8.74 mg N/1, the recoveries were 99 and 99%, respectively.

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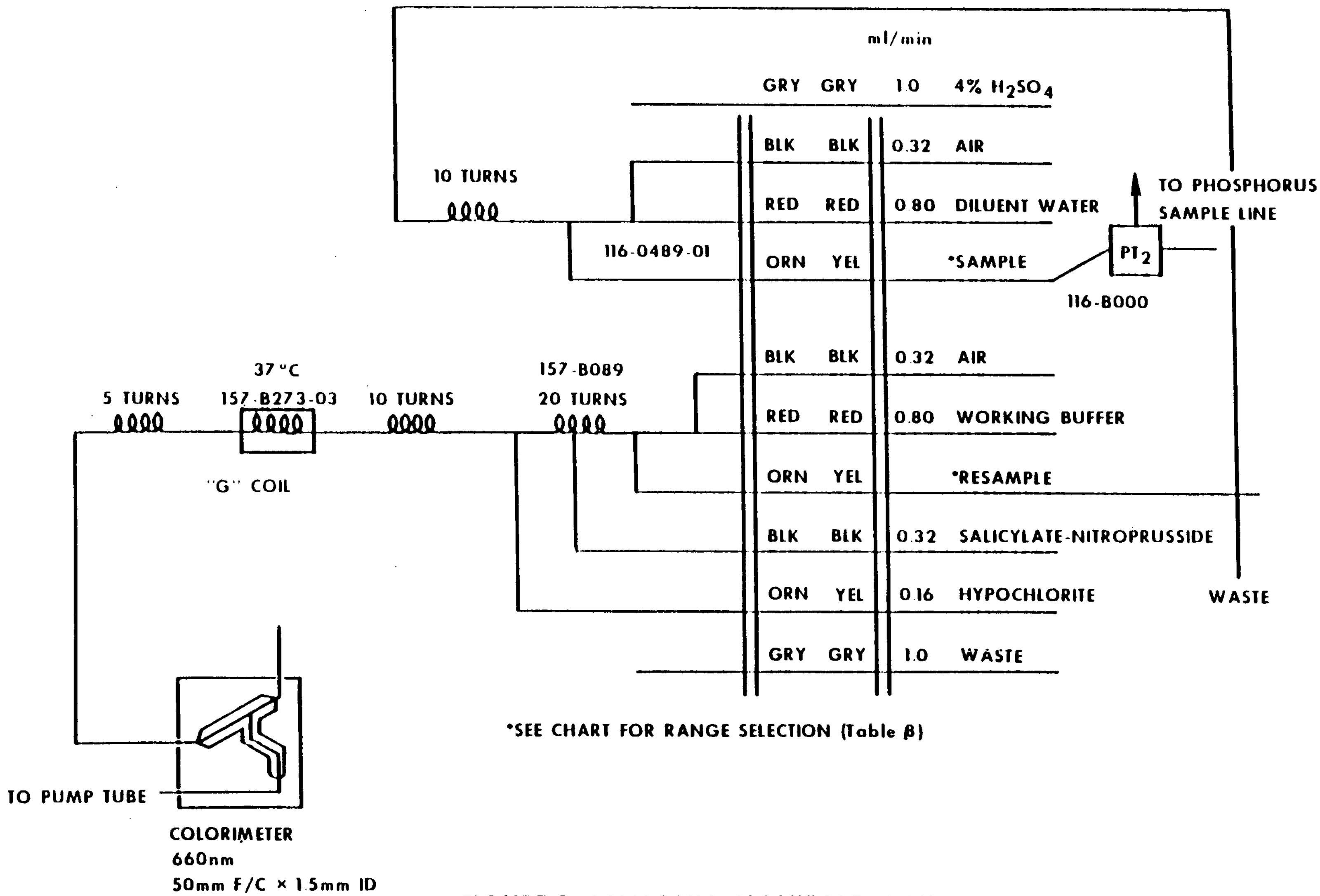

FIGURE 1. AMMONIA MANIFOLD AAII

QuikChem METHOD 10-107-06-2-D

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS

6645 WEST MILL ROAD

MILWAUKEE, WI 53218, USA

LACHAT
INSTRUMENTS

QuikChem Method 10-107-06-2-D

Total Kjeldahl Nitrogen in Waters

0.2 to 20 .0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit

2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)

3. 5 mL and 20 mL Repipet Dispensers

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DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.

1.3. The applicable range is 0.2 to 20 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. The sample is heated in the presence of sulfuric acid, H_2SO_4, for two and one half hours. The residue is cooled, diluted with water an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.

2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of

calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1, 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation, the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem, the buffer can also be degasssed.

Reagent 1. **Mercuric Sulfate Solution**

To a 100 mL volumetric flask add approximately 40.0 mL water and **10 mL** concentrated **sulfuric acid** (H_2SO_4). Then add **8.0 g red mercuric oxide** (HgO). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

Reagent 2. **Digestion Solution**

In a 1 L volumetric flask, add **133.0 g potassium sulfate** (K_2SO_4) and **200 mL concentrated sulfuric acid** (H_2SO_4) to approximately **700 mL water**. Add **25.0 mL Reagent 1**. Dilute to the mark with water and invert to mix. Prepare fresh monthly.

Reagent 3. **Buffer**

By Volume: In a 1 L volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. **Salicylate Nitroprusside**

By Volume: In a **1 L** volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about **800 mL water**. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

By Weight: To a tared **1 L** dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] and **908 g water**. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.

Reagent 5. **Hypochlorite Solution**

By Volume: In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

By Weight: To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. **Diluent 5.0% (V/V) Sulfuric Acid**

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved. standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 1000 mg N/L

In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific. cat. no. A938-500.

Standard 2. Working Stock Standard 20.0 mg N/L

By Volume: In a **250 mL** volumetric flask. dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	125	62.5	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**. add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared 1 L container. add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (20.0 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**. add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (1000 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20** g of Standard 1 (1000 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	20.00	10.00	5.00	2.00	1.00	0.500	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with Reagent 6	100	50	25	10	5	2.5	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with Reagent 6.	250.0	125	62	25	12.5	6.25	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.50	0.25	0.10	0.05	0.025	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to pH < 2 and cooled to 4^{O}C at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4^{O}C and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being > 10X MDL. If any determined concentration exceeds the known values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going

precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration. after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_S - C}{s} \ X100$$

Where,

R = percent recovery

C_S = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar granules are available from Fisher Scientific, cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy." 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and

by complying with all solid and hazardous waste regulations. and by complying with all solid and hazardous waste regulations. particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 351.2

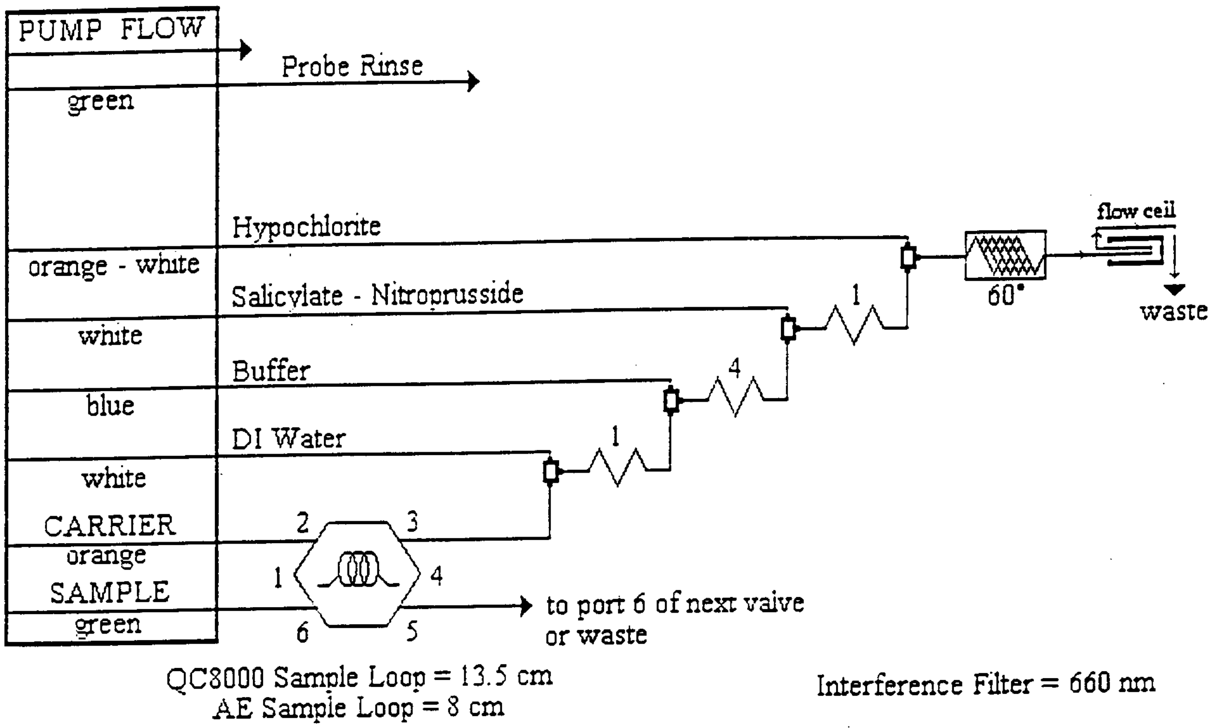
2. ASTM, Water(I), Volume 11.01. Method D3590-89. Test Methods for Kjeldahl Nitrogen in Water, p. 447

3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes**, EPA-600/4-79-020, Revised March 1983, Method 350.1

4. Code of Federal Regulations 40. Chapter 1, Part 136. Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

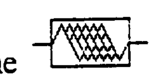
17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve, flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d.** This is **5.2 uL/cm.**

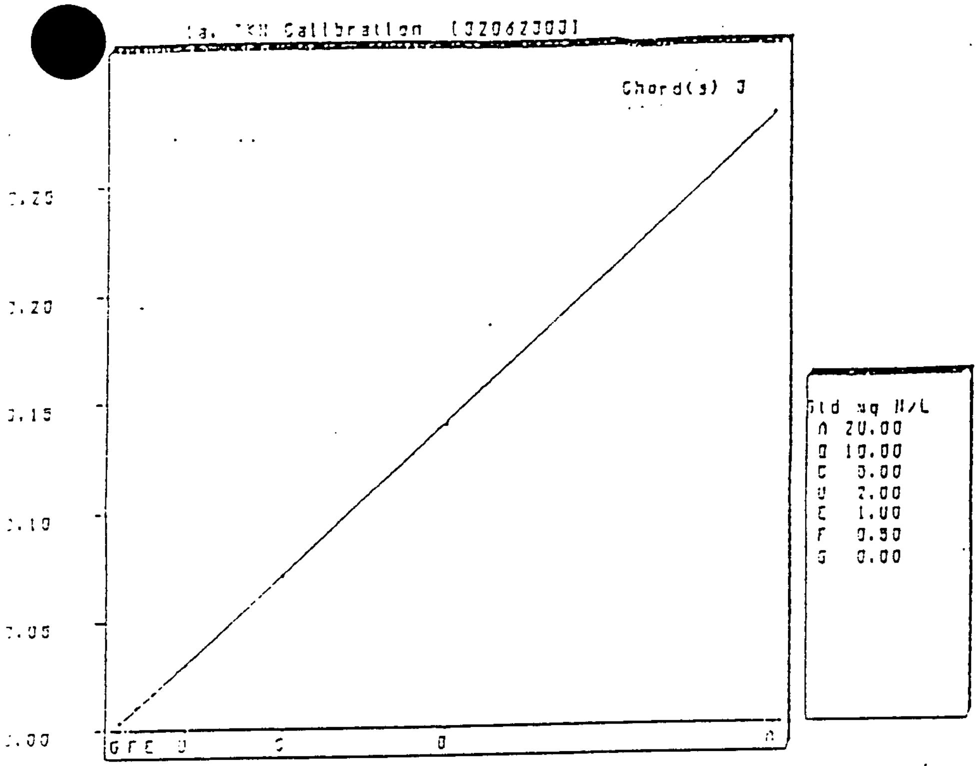
MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2 DATA SYSTEM PARAMETERS FOR THE QUIKCHEM AE

Sample throughput:		90 samples/hour: 60 s/sample
Pump speed:		35
Cycle Period:		45 s
Inject to start of peak period:		38 s
Presentation. Data Window		
Top Scale Response:		0.32 abs
Bottom Scale Response:		0.00 abs
Segment/Boundaries:	A:	20.00 mg N/L
	E:	1 mg N/L
	F:	0.00 mg N/L

Series 4000/System IV Settings: Gain = 420 x 1

17.3 QUIKCHEM AE SUPPORT DATA



Calibration Statistics Report

Cal Ref: 92062303 06/24/92 03:48 pm
Method: TKN-F

Channel: TKN

Correlation Coefficients

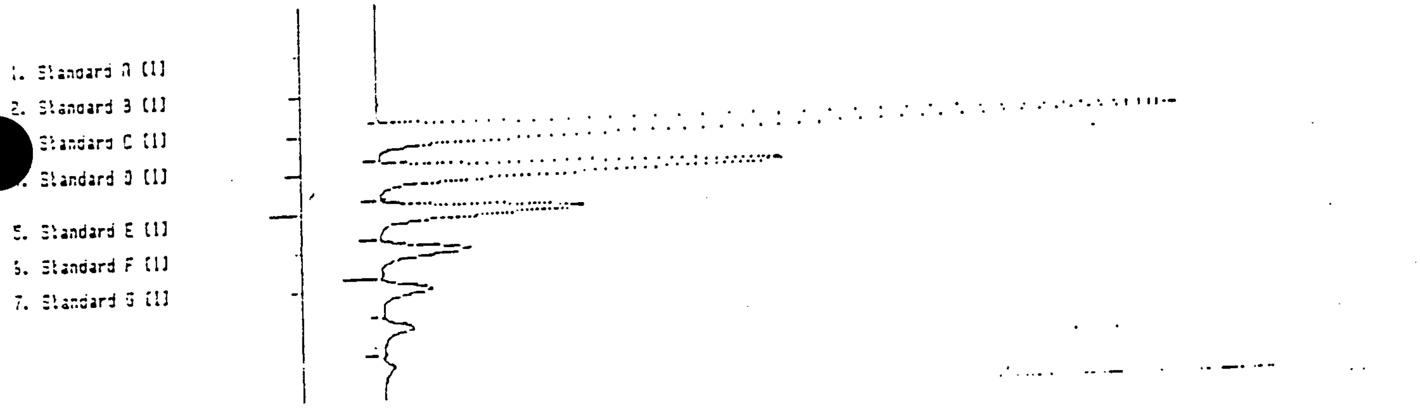
Seg Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1 A-E	0.9999	0.9992	1.0000	1.0000	0.9999	0.4213 ?
2 E-G	0.9979	0.7702 ?	1.0000	1.0000	0.9991	0.5000 ?

Percent Standard Deviation in Slope

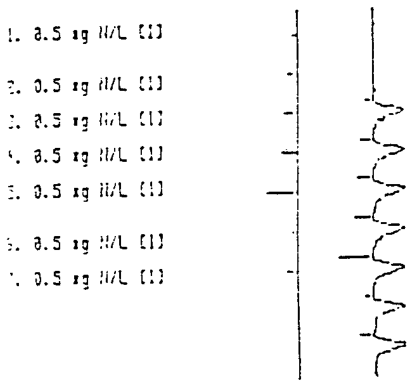
1 A-E	0.7	1.6	0.2	0.1	0.6	97.
2 E-G	4.6	31.5 ?	0.0	0.0	4.3	72.

QuikChem AE Calibration Report for Calibration 92062303
Method: TKN
This calibration was done on 06/23/92 at 07:51 pm
This report prepared on 06/24/92 at 03:44 pm

	Analyte	Units	Average Concentrations Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A,	TKN	mg N/L	20.000	19.812	-0.21	0.2795
Standard B,	TKN	mg N/L	10.000	9.913	0.37	0.1374
Standard C,	TKN	mg N/L	5.000	4.994	0.12	0.0714
Standard D,	TKN	mg N/L	2.000	2.997	-0.34	0.0301
Standard E,	TKN	mg N/L	1.000	1.000	0.09	0.0168
Standard F,	TKN	mg N/L	0.500	0.500	-0.41	0.0101
Standard G,	TKN	mg N/L	0.000	-0.080	[illegible]	0.0033



ack 1 (Ref: 92062308) 06/23/1992, 08:08 pm

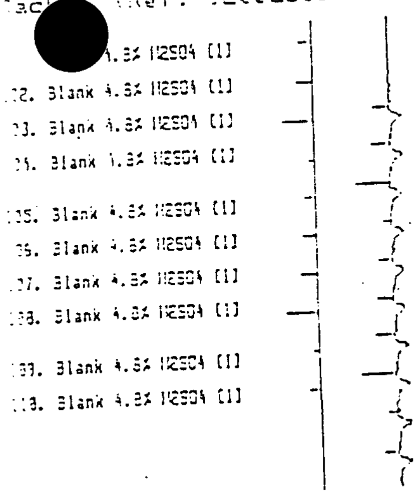


Cup#	Sample ID	1 TKN mg N/L
101	0.5 mg N/L	0.492<
102	0.5 mg N/L	0.496<
103	0.5 mg N/L	0.506
104	0.5 mg N/L	0.492<
105	0.5 mg N/L	0.479<
106	0.5 mg N/L	0.487<
107	0.5 mg N/L	0.503

MDL

mean = 0.494
s = 0.0092
(3.14) s = 0.029 mg N/L

Rack (Ref: 92062306) 06/23/1992, 07:51 pm

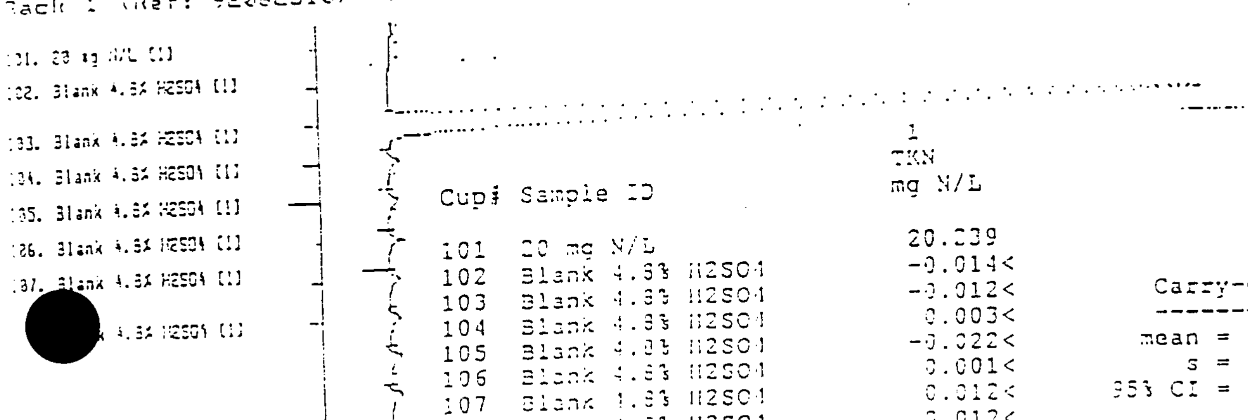


Cup#	Sample ID	1 TKN mg N/L
101	Blank 4.8% H2SO4	0.010<
102	Blank 4.8% H2SO4	-0.004<
103	Blank 4.8% H2SO4	0.007<
104	Blank 4.8% H2SO4	0.012<
105	Blank 4.8% H2SO4	-0.003<
106	Blank 4.8% H2SO4	-0.000<
107	Blank 4.8% H2SO4	0.021<
108	Blank 4.8% H2SO4	0.008<
109	Blank 4.8% H2SO4	0.015<
110	Blank 4.8% H2SO4	-0.007<

EMDL DATA

Mean = 0.0059
s = 0.0091
(4.65) s = 0.04 mg N/L

Rack 1 (Ref: 92062310) 06/23/1992, 08:29 pm

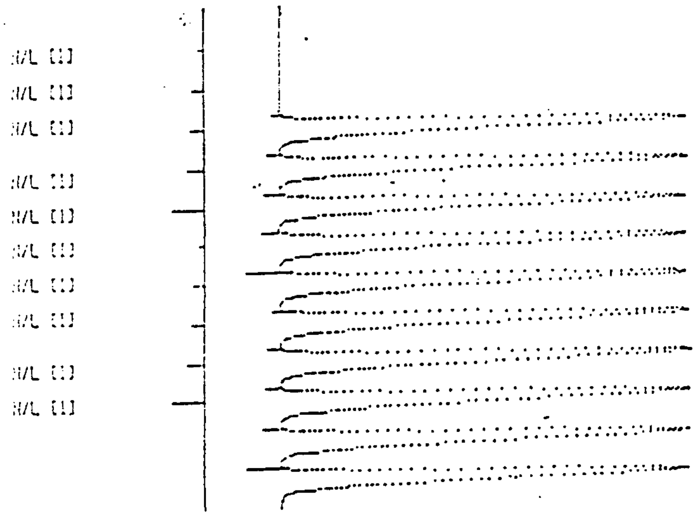


Cup#	Sample ID	1 TKN mg N/L
101	20 mg N/L	20.239
102	Blank 4.8% H2SO4	-0.014<
103	Blank 4.8% H2SO4	-0.012<
104	Blank 4.8% H2SO4	0.003<
105	Blank 4.8% H2SO4	-0.022<
106	Blank 4.8% H2SO4	0.001<
107	Blank 4.8% H2SO4	0.012<
108	Blank 4.8% H2SO4	0.012<

Carry-over

mean = -0.0029
s = 0.0133
95% CI = -0.015 to 0.009
(passes)

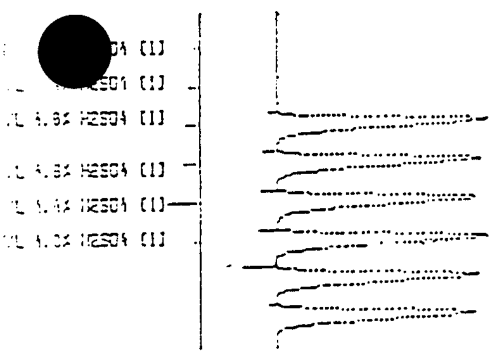
(Ref: 92062309) 06/23/1992, 08:15 pm



Cup#	Sample ID	1 TKN mg N/L
101	10.0 mg N/L	10.017
102	10.0 mg N/L	10.022
103	10.0 mg N/L	9.971
104	10.0 mg N/L	9.989
105	10.0 mg N/L	10.001
106	10.0 mg N/L	10.096
107	10.0 mg N/L	10.126
108	10.0 mg N/L	10.102
109	10.0 mg N/L	10.058
110	10.0 mg N/L	10.065

Precision
----
mean = 10.06
s = 0.065
%RSD = 0.64

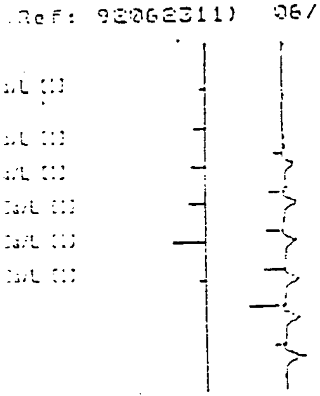
(Ref: 92062307) 06/23/1992, 08:00 pm



Cup#	Sample ID	1 TKN mg N/L
101	5.0 mg N/L 4.8% H2SO4	5.039
102	5.0 mg N/L 4.4% H2SO4	4.893
103	5.0 mg N/L 4.0% H2SO4	4.753
104	5.0 mg N/L 4.8% H2SO4	5.011
105	5.0 mg N/L 4.4% H2SO4	4.825
106	5.0 mg N/L 4.0% H2SO4	4.757

Acid Effect
----
mean (4.8%) = 5.025 mg N/L
mean (4.4%) = 4.859 mg N/L
mean (4.0%) = 4.775 mg N/L

(Ref: 92062311) 06/23/1992, 08:38 pm



Cup#	Sample ID	1 TKN mg N/L
101	20 mg Ca/L	-0.002<
102	20 mg Ca/L	0.001<
103	20 mg Ca/L	0.021<
104	100 mg Ca/L	0.044<
105	100 mg Ca/L	0.051<
106	100 mg Ca/L	0.080<

Calcium Interference

<0.10 mg N/L at 100 mg Ca/L

17.4 DATA PARAMETERS FOR THE QUIK CHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width: 39 s

% Width Tolerance: 100

Threshold: 11537

Inject to Peak Start: 42 s

Chemistry: Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	20.00	10.00	5.00	2.00	1.00	0.50	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

Sampler Timing:

Min. Probe in Wash Period: 14 s

Probe in Sample Period: 20 s

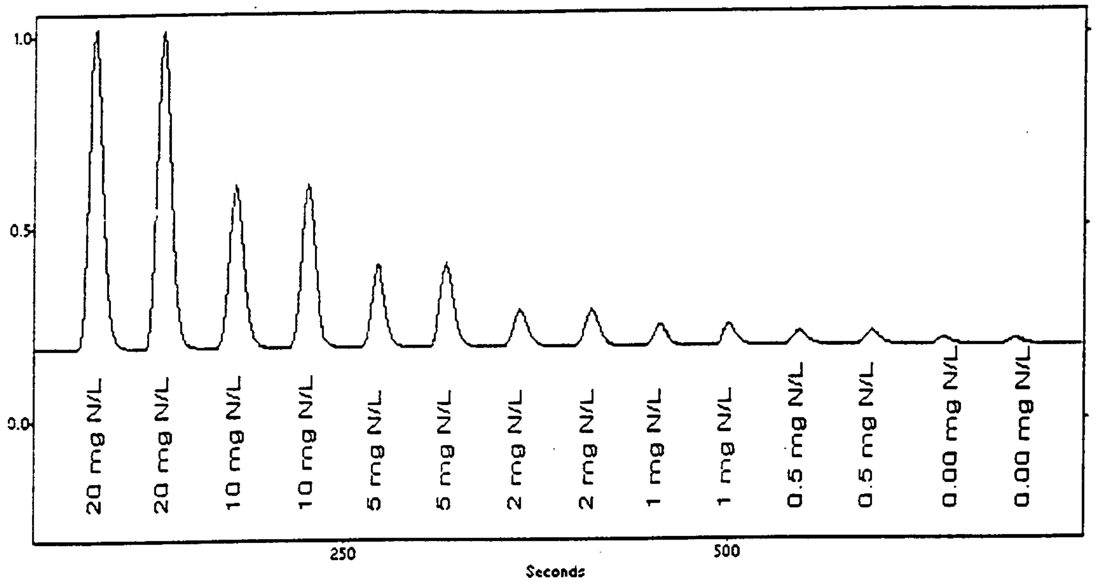
Valve Timing:

Load Period: 20 s

Inject Period: 25 s

Load Time: 0.0 s

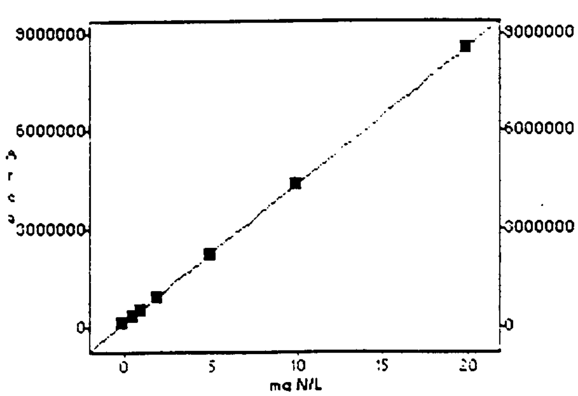
17.5 QUIKCHEM 8000 SUPPORT DATA



ACQ. TIME: Aug 22, 1994 13:25:57
DATA FILENAME: C:\OMNION\DATA\1010762E\082294C2.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

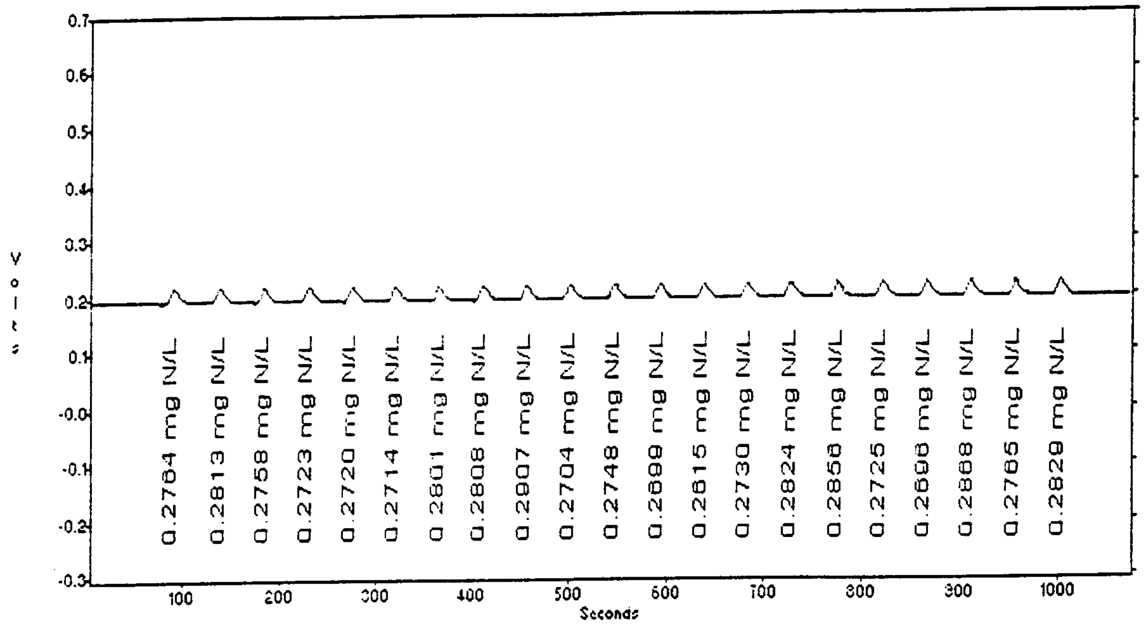
Calibration Graph and Statistics

Level	Area	mg CN⁻/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8596849	20	20.000	8596849	8634613	26703.2	0.3	-0.0
2	4383597	10	10.020	438597	4373046	7460.7	0.2	-0.2
3	2248447	5	4.960	2248447	2246723	1218.7	0.1	0.8
4	990856	2	1.991	990856	978804	8522.1	0.9	0.9
5	574638	1	0.997	574638	566821	5527.9	1.0	0.3
6	366814	0.5	0.504	366814	364718	1481.9	0.4	-0.9
7	167977	0	0	167977	165993	1403.2	0.8	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 2.369e-006Area-3.646e-001
$R^2 = 1.000$

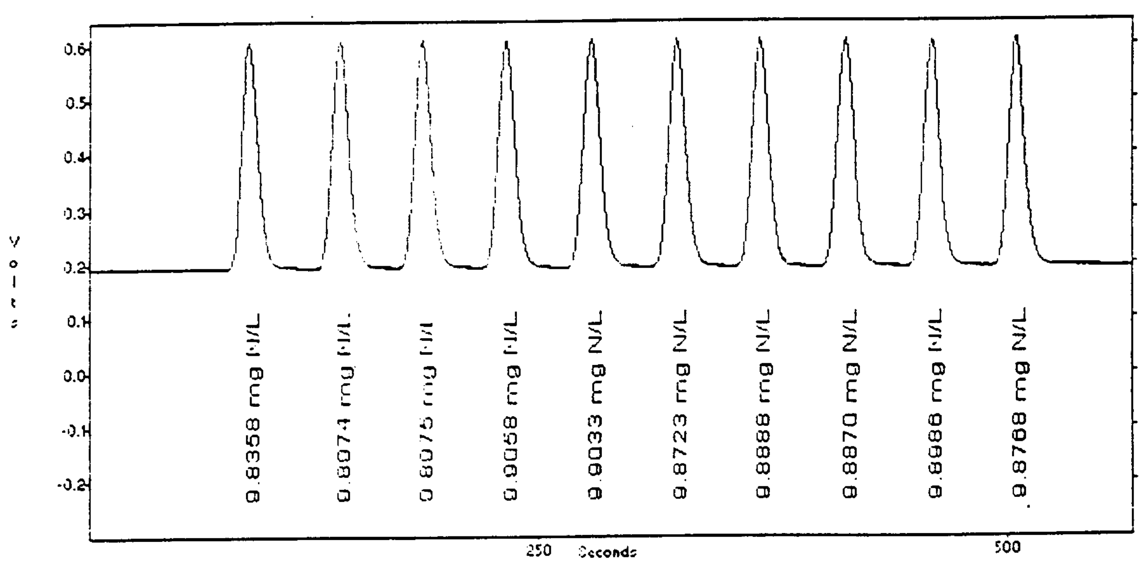
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME:	Aug 22, 1994 13:54:02
DATA FILENAME:	C:\OMNION\DATA\1010762E\082294M1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762D\1010762D.MET

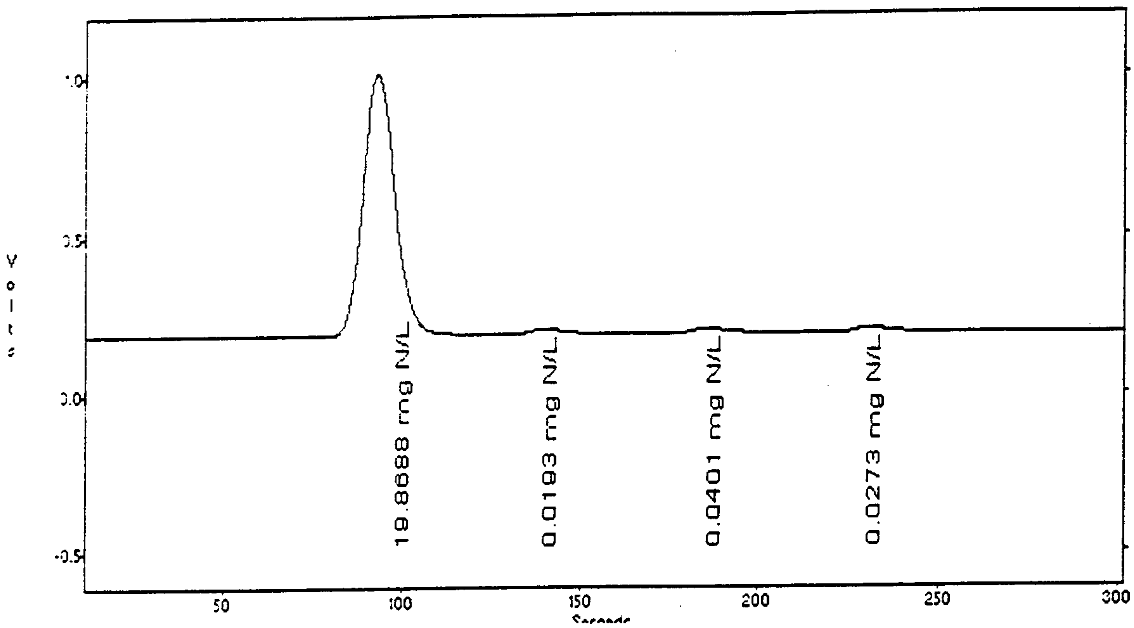
Figure 3. Precision



Precision = 0.211 % RSD

ACQ. TIME:	Aug 22, 1994 14:21:28
DATA FILENAME:	C:\OMNION\DATA\1010762E\082294P1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762D\1010762D.MET

Figure 4. Carryover



ACQ. TIME: Aug 22, 1994 14:32:42
DATA FILENAME: C:\OMNION\DATA\1010762E\082294R1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762D\1010762D.MET

QuikChem METHOD 10-107-06-2-E

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY

(BLOCK DIGESTOR METHOD)

Written by David H. Diamond

Applications Group

Revision Date:

18 October 1994

LACHAT INSTRUMENTS

6645 WEST MILL ROAD

MILWAUKEE, WI 53218, USA

LACHAT

INSTRUMENTS

QuikChem Method 10-107-06-2-E

Total Kjeldahl Nitrogen in Waters

0.1 to 5.0 mg N/L

-- Principle --

This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

-- Interferences --

1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.4% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

2. High nitrate cocncentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

-- Special Apparatus --

1. Heating Unit

2. Block Digestor/75 mL tubes (Lachat Part. No. 1800-000)

3. 5 mL and 20 mL Repipet Dispensers

Revised by Karin Wendt 18 October 1994 written by D. Diamond and copyrighted on 24 June 92 by **Lachat Instruments, 6645 West Mill Road, Milwaukee, WI 53218, USA.** Phone: (414) 358-4200 FAX: 1-414-358-4206.

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DETERMINATION OF TOTAL KJELDAHL NITROGEN BY FLOW INJECTION ANALYSIS COLORIMETRY (BLOCK DIGESTOR METHOD)

1. SCOPE AND APPLICATION

1.1. The method covers the determination of total Kjeldahl nitrogen in water and wastewater.

1.2. The colorimetric method is based on reactions that are specific for the ammonia ion. The digestion converts organic forms of nitrogen to the ammonium form. Nitrate is not converted to ammonium during digestion.

1.3. The applicable range is 0.1 to 5 mg N/L. The method detection limit is 0.02 mg N/L. 90 samples per hour can be analyzed.

1.4. Samples containing particulates should be filtered or homogenized.

2. SUMMARY OF METHOD

2.1. The sample is heated in the presence of sulfuric acid, H_2SO_4, for two and one half hours. The residue is cooled, diluted with water an analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of the digestion described.

2.3. Organic nitrogenous the difference obtained by subtracting the free-ammonia concentration from the total Kjeldahl nitrogen concentration.

2.4. Approximately 0.1 mL of the digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH by neutralization and with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and also prevents undue influence of the sulfuric acid matrix on the pH-sensitive color reaction which follows.

2.5. The ammonia thus produced is heated with salicylate and hypochlorite to produce blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards. but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample. and its purpose is to determine whether the methodology is in control. and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like sample. and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LSM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that is digested exactly as a sample including exposure to all glassware. equipment. and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents. or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity. health hazards, physical properties. fire, and reactivity data including storage. spill. and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified. measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of

calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

4.1. Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 5.0 to 4.5% (v/v) H_2SO_4 in the diluted digestion sample with no change in signal intensity.

4.2. High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected. samples should be diluted and reanalyzed.

4.3. Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous. consult MSDS.

5.3.1. Mercury (Reagents 1 and 2)

5.3.2. Sulfuric Acid (Reagents 1. 2 and 6)

5.3.3. Sodium Nitroprusside (Reagent 4)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical. capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part. No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with Helium

To prevent bubble formation. the water carrier is degassed with helium. Use He at 20 lb/in^2 through a helium degassing wand. Bubble He vigorously through the solution for one minute. If air spikes continue to be a problem. the buffer can also be degasssed.

Reagent 1. Mercuric Sulfate Solution

To a 100 mL volumetric flask add approximately 40.0 mL water and **10 mL** concentrated **sulfuric acid** (H_2SO_4). Then add **8.0 g red mercuric oxide** (HgO). Stir until dissolved, dilute to the mark and invert to mix. Warming the solution while stirring may be required to dissolve the mercuric oxide.

Reagent 2. Digestion Solution

In a 1 L volumetric flask, add **133.0 g potassium sulfate** (K_2SO_4) and **200 mL concentrated sulfuric acid** (H_2SO_4) to approximately **700 mL water**. Add **25.0 mL Reagent 1**. Dilute to the mark with water and invert to mix. Prepare fresh monthly.

Reagent 3. Buffer

By Volume: In a **1 L** volumetric flask containing **900 mL water** completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH), dilute to the mark and invert to mix. Degas weekly and prepare fresh monthly.

By Weight: To a tared **1 L** container add **958 g water** and completely dissolve **30.0 g sodium phosphate dibasic heptahydrate** ($Na_2HPO_4 \cdot 7H_2O$). Next, add **17.0 g disodium EDTA** (ethylenediaminetetracetic acid disodium salt). The EDTA will not dissolve but will form a turbid solution. Finally, add **65 g sodium hydroxide** (NaOH). Stir or shake until dissolved. Degas weekly and prepare fresh monthly.

Reagent 4. Salicylate Nitroprusside

By Volume: In a **1 L** volumetric flask dissolve **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], and **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about **800 mL water**. Dilute to the mark and invert to mix. Store in a dark bottle and prepare fresh monthly.

By Weight: To a tared 1 L dark container, add **150.0 g sodium salicylate** [salicylic acid sodium salt, $C_6H_4(OH)(COO)Na$], **1.00 g sodium nitroprusside** [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] and **908 g water**. Stir or shake until dissolved. Store in a dark bottle and prepare fresh monthly.

Reagent 5. **Hypochlorite Solution**

By Volume: In a **250 mL** volumetric flask, dilute **15.0 mL Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) to the mark with water. Invert to mix. Prepare fresh daily.

By Weight: To a tared **250 mL** container, add **16 g of Regular Clorox Bleach** (5.25% sodium hypochlorite, The Clorox Company, Oakland, CA) and **234 g DI water**. Shake to mix. Prepare fresh daily.

Reagent 6. **Diluent 5.0% (V/V) Sulfuric Acid**

NOTE: Diluent is prepared to dilute off scale samples. This reagent is not used on-line.

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

7.2. PREPARATION OF STANDARDS

Prepare standards in DI water daily or preserve them with 2 mL/L sulfuric acid. Once preserved, standards may be stored for 28 days. Standards in digest matrix may be stored for up to 28 days. If samples always fall within a narrower range, more standards within this narrower range can be added and standards outside this narrower range can be dropped.

Digested Standards

NOTE: Working standards prepared in DI water are digested per the procedure in section 8.

Standard 1: Stock Standard 250 mg N/L

In a **1 L** volumetric flask dissolve **0.9540 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about **800 mL DI water**. Dilute to the mark and invert to mix. As an alternative, primary standard grade ammonium sulfate is available from Fisher Scientific, cat. no. A938-500 (use 1.18g).

Standard 2. Working Stock Standard 5.0 mg N/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard 1** to the mark with **DI water**. Invert to mix.

By Weight: To a tared 1 L container add about **20 g Stock Standard 1**. Divide the exact weight of the standard solution by **0.02** and dilute up to this resulting total weight with **DI water**. Shake to mix.

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 2** diluted to 100 mL with DI water	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 2** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

Non-Digested Standards

Standard 3. Blank in Digestion Matrix (0.00 mg N/L)

By Volume: In a 1 L volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **250 mL Reagent 2 (Digestion Solution)**. Invert to mix.

Standard 4. High Standard in Digestion Matrix (5.00 mg N/L)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water**, add **250 mL Reagent 2** (Digestion Solution). Add **20 mL** of Standard 1 (250 mg N/L). Allow the solution to cool and dilute to the mark with DI water. Invert to mix. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **740 g water** and **250 mL Reagent 2 (Digestion Solution)**. Add **20 g** of Standard 1 (250 mg N/L) and shake to mix.

Note: Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg N/L for a 5.00 mg N/L non-digested standard. If non-digested standards are used to calibrate, the "labeled" concentrations should be entered in the data system.

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

Preparation of Non-digested Working Standards

Working Standards Prepare Daily)	A	B	C	D	E	F	G
Concentration mg N/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

By Volume

Volume (mL) of **Standard 4** diluted to 100 mL with **Standard 3**	100	40	20	10	5	2	0

By Weight

Weight (g) of **Standard 4** diluted to final weight (~250 g) divide by **factor** below with **Standard 3**.	250.0	100	50	25	12.5	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.05	0.02	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with dilute hydrochloric acid (0.5 M) and then rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis and minimize waste disposal.

8.2. Samples should be preserved to pH < 2 and cooled to 4°C at the time of collection.

8.3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

9. QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards, the lowest concentration being > 10X MDL. If any determined concentration exceeds the known values by +/-10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) spiked at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the spiked reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates].

S = standard deviation of the replicate analyses.

MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Spiked Blank (LSB) -- The laboratory must analyze at least one LSB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LSB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going

precision statement for the level of concentrations included in the LSB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LSM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory spiked blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unspiked sample, and compare these values to the designated LSM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_S - C}{s} \ X100$$

Where,

R = percent recovery

C_S = spiked sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of 7 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in section 7.2).

10.4. Calibrate the instrument as description in section 11.

10.2. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.3. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

NOTE: Some laboratories prepare standards in DI water and process them through the digestion as outlined below. Other laboratories calibrate using standards in the digest matrix, i.e., **NOT** digested. Instructions for preparing standards in the digest matrix are given in section 7 of this method, following the instructions for preparing standards in DI water. At a minimum, two blanks and one standard should be prepared in DI water and digested.

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To 20.0 mL of sample or standard add 5 mL digestion solution and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add 2 - 4 Hengar granules or 10 - 12 teflon stones to each tube. Hengar (Alundum) granules and teflon stones are effective for smooth boiling. Hengar

granules are available from Fisher Scientific. cat. no. S145-500. Teflon stones are available from Markson Science, cat. no. 248-808, (800) 528-5114.

11.1.4. Ensure that the digestion tubes are dry on the outside and that all tubes contain boiling stones. Verify that boiling stones have been placed in each tube. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 5.

11.1.5. Continue to digest for 1.5 additional hours with the controller set to 380°C. This time includes the ramp time for the block temperature to come up to 380°C. The typical ramp time is 50 - 60 minutes. 380°C must be maintained for 30 minutes.

11.1.6. Before removing samples, gather the necessary supplies to dilute the samples with water. Remove the samples from the block and allow exactly 5 minutes to cool. Add water to the samples rapidly so that all samples are diluted within 10 minutes of removal from the block.

11.1.7. Add 19.0 mL DI water to each tube and vortex to mix. The total final volume should be 20 mL. The longer the samples have been allowed to cool, the longer the samples should be vortexed. For samples diluted at 5 minutes, 10 seconds of vortexing is sufficient. For samples which have cooled for greater than 10 minutes, up to 30 seconds of vortexing may be necessary.

11.1.8. If samples are not run immediately they should be diluted, vortexed and covered with lab film or capped tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.1.

11.2.3. Input peak timing and integration window parameters as specified in section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the autosampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis (please refer to system notes).

11.4. SYSTEM NOTES

11.4.1. Allow at least 15 minutes for the heating unit to warm up to 60°C.

11.4.2. If sample concentrations are greater than the high standard the digested sample should be diluted with **Reagent 6**. When the digital diluter is used, **Reagent 6** should be used as diluent. Do not dilute digested samples or standards with **DI water**.

11.4.3 If the salicylate reagent is merged with a sample containing sulfuric acid in the absence of the buffer solution, the salicylate reagent will precipitate. If this occurs all teflon manifold tubing should be replaced. To prevent this, prime the system by first placing the buffer transmission line in the buffer. Pump until the air bubble introduced during the transfer reaches the "T" fitting on the manifold. Then place all other transmission lines in the proper containers.

11.4.4. In normal operation nitroprusside gives a yellow background color which combines with the blue indosalicylate to give an emerald green color. This is the normal color of the solution in the waste container.

11.4.5. In normal operation the digest blank will result in a peak of about 1/5 the area of the 0.5 mg N/L standard. This peak is due to the acid in the digest and is present in every injection. Since this blank is constant for all samples and standards it will not effect data quality.

11.4.6. If phosphorus is also determined with the Lachat System, a second helium degassing tube should be purchased and the tubes should be dedicated to the individual chemistries.

11.4.7. If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure:

A. Place transmission lines in water and pump to clear reagents (2-5 minutes).

B. Place reagent lines in **1 M hydrochloric acid** (1 volume of HCl added to 11 volumes of water) and pump for several minutes.

C. Place all transmission lines in water and pump for several minutes.

D. Resume pumping reagents.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply the answer by the appropriate dilution factor.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in mg N/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in section 19.2. This data was generated according to Lachat Standard Operating Procedure J001. Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's Department of Government Regulations and Science Policy," 115 16Th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The Environmental Protection Agency (USEPA) requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the

"Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

1. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes.** EPA-600/4-79-020. Revised March 1983. Method 351.2

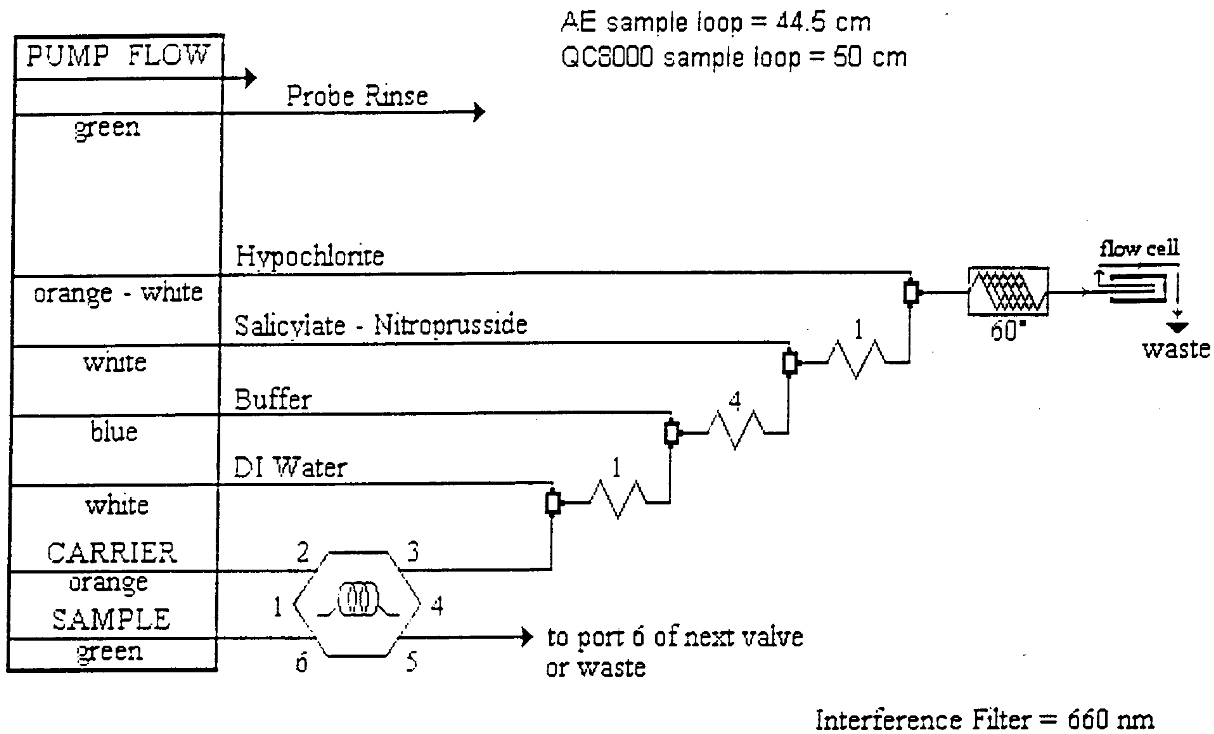
2. ASTM. Water(I), Volume 11.01. Method D3590-89. Test Methods for Kjeldahl Nitrogen in Water. p. 447

3. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes.** EPA-600/4-79-020. Revised March 1983. Method 350.1

4. Code of Federal Regulations 40. Chapter 1. Part 136. Appendix B.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. TOTAL KJELDAHL NITROGEN MANIFOLD DIAGRAM:



CARRIER is helium degassed water.

1 is **70 cm** of tubing on a **1 inch** coil support

4 is **255 cm** of tubing on a **4 inch** coil support

Apparatus: Standard valve, flow cell, and detector head modules are used. The  shows **650 cm of heated tubing**. All manifold tubing is **0.8 mm (0.032 in) i.d.** This is **5.2 uL/cm.**

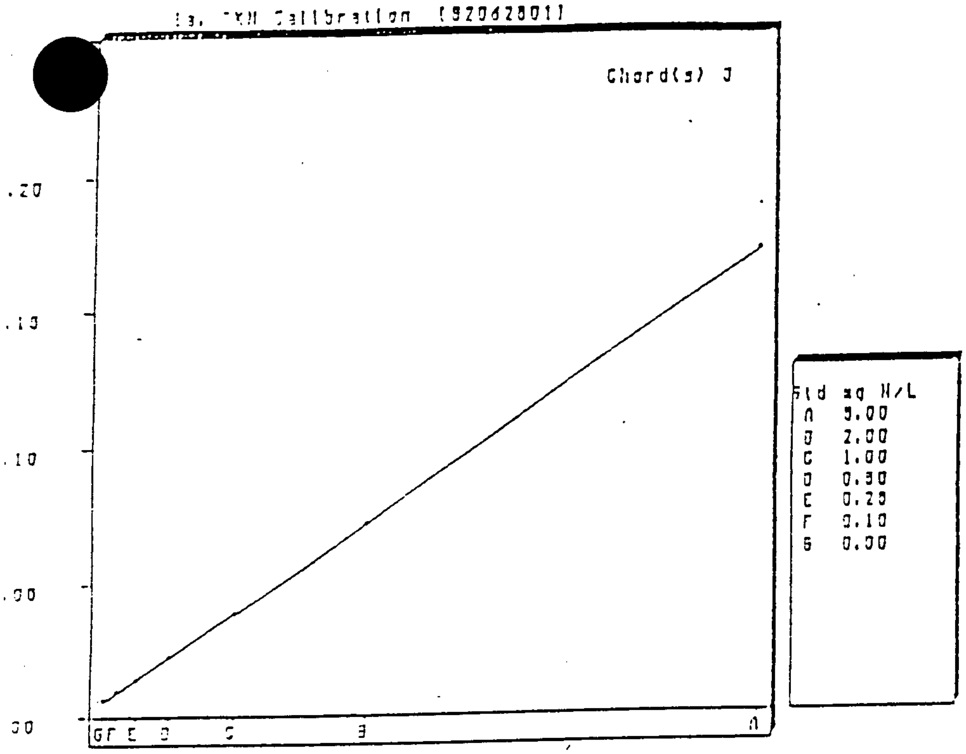
MANIFOLD DIAGRAM REVISION DATE: 15 July 1992 by D. Diamond - 26Jul94 lc

17.2. DATA SYSTEM PARAMETERS FOR QUIKCHEM AE

Sample throughput:		90 samples/hour: 60 s/sample
Pump speed:		35
Cycle Period:		45 s
Inject to start of peak period:		38 s
Presentation. Data Window		
Top Scale Response:		0.25 abs
Bottom Scale Response:		0.00 abs
Segment/Boundaries:	A:	5.00 mg N/L
	C:	1 mg N/L
	G:	0.00 mg N/L

Series 4000/System IV Settings: Gain = 570 x 1

17.3. QUIKCHEM AE SUPPORT DATA



CalibrationStatistics Report

Cal Ref: 92062501 06/25/92 01:43 pm

Method: TKN-F

Channel: TKN

Correlation Coefficients

Seg	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-C	1.0000	0.9992	0.9997	1.0000	0.9999	1.0000
2	C-G	0.9986	0.9384 ?	0.9980	0.9998	0.9959	0.4112 ?

Percent Standard Deviation In Slope

1	A-C	0.3	2.0	1.8	0.5	0.0	0.4
2	C-G	2.2	25.3	2.6	0.8	3.7	61.1 ?

QuikChem AE Calibration Report for Calibration 92062501

Method: TKN

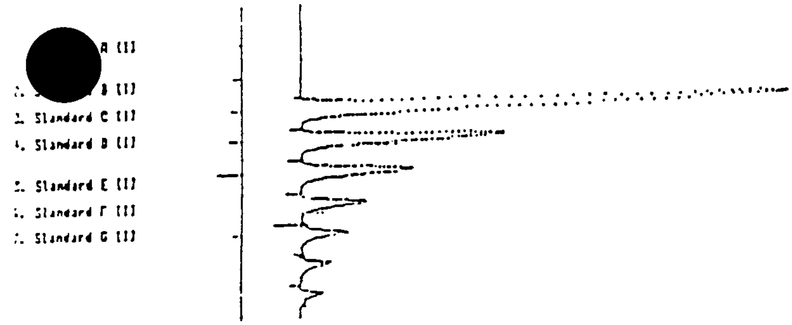
This calibration was done on 06/25/92 at 12:25 pm

This report prepared on 06/25/92 at 01:31 pm

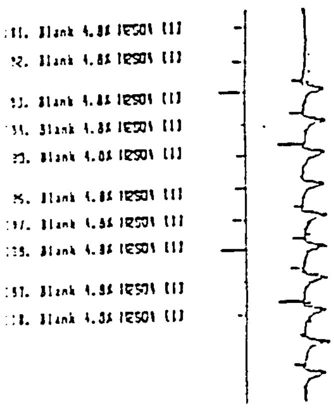
Analyte	Units	Average Concentrations: Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A, TKN	mg N/L	5.000	5.003	-0.05	0.1715
Standard B, TKN	mg N/L	2.000	1.989	0.54	0.0711
Standard C, TKN	mg N/L	1.000	1.004	-0.36	0.0345
Standard D, TKN	mg N/L	0.500	0.496	0.78	0.0229
Standard E, TKN	mg N/L	0.250	0.242	3.27	0.0137
Standard F, TKN	mg N/L	0.100	0.102	-1.77	0.0072
Standard G, TKN	mg N/L	0.000	0.006	***.**	0.0061

End of Calibration Report For Calibration 92062501

Calibration 06/25/1992, 12:17 pm



Rack 1 (Ref: 92062502) 06/25/1992, 12:25 pm



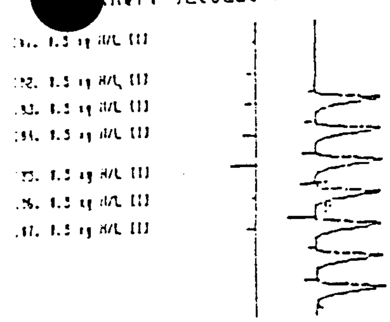
Cup#	Sample ID	1 TKN mg N/L
101	Blank 4.0% H2SO4	0.013<
102	Blank 4.0% H2SO4	0.012<
103	Blank 4.0% H2SO4	0.009<
104	Blank 4.0% H2SO4	0.003<
105	Blank 4.0% H2SO4	0.049<
106	Blank 4.0% H2SO4	-0.007<
107	Blank 4.0% H2SO4	0.010<
108	Blank 4.0% H2SO4	0.009<
109	Blank 4.0% H2SO4	0.009<
110	Blank 4.0% H2SO4	-0.013<

EMDL Determination

mean = 0.009
s = 0.015
4.65 (s) = 0.076 mg N/L

--------- End of Report for Tray 92062502.RS -----------

(Ref: 92062504) 06/25/1992, 12:41 pm



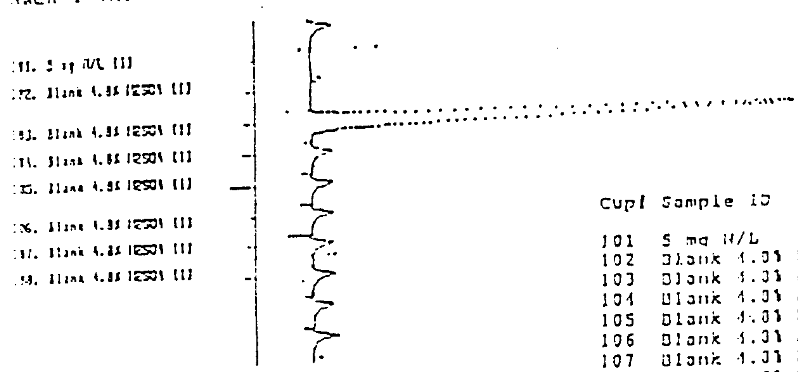
Cup#	Sample ID	1 TKN mg N/L
101	0.5 mg N/L	0.489
102	0.5 mg N/L	0.486
103	0.5 mg N/L	0.488
104	0.5 mg N/L	0.491
105	0.5 mg N/L	0.470
106	0.5 mg N/L	0.492
107	0.5 mg N/L	0.490

HDL Study

mean = 0.487
s = 0.008
(3.14) s = 0.024

--------- End of Report for Tray 92062504.RS -----------

Rack 1 (Ref: 92062506) 06/25/1992, 12:54 pm



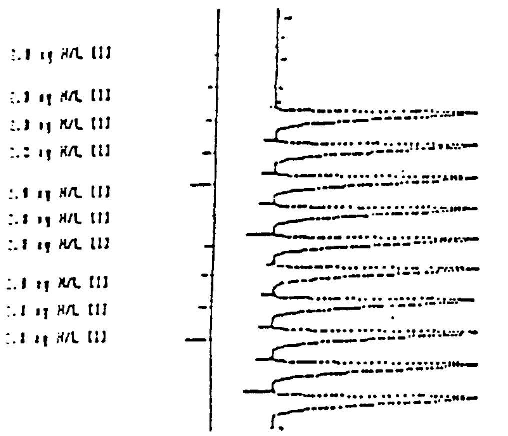
Cup#	Sample ID	1 TKN mg N/L
101	5 mg N/L	4.852
102	Blank 4.0% H2SO4	0.003<
103	Blank 4.0% H2SO4	0.002<
104	Blank 4.0% H2SO4	0.001<
105	Blank 4.0% H2SO4	-0.001<
106	Blank 4.0% H2SO4	0.009<
107	Blank 4.0% H2SO4	0.004<
108	Blank 4.0% H2SO4	-0.009<

Carry-over Study

mean = 0.001
s = 0.005
95% CI (-0.004 to 0.006)
passes

--------- End of Report for Tray 92062506.RS -----------

1 (Ref: 92062507) 06/25/1992, 01:04 pm



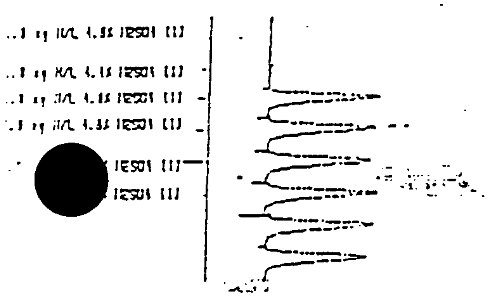
Cup	Sample ID	1 TKN mg N/L
101	2.0 mg N/L	1.923
102	2.0 mg N/L	1.926
103	2.0 mg N/L	1.925
104	2.0 mg N/L	1.926
105	2.0 mg N/L	1.947
106	2.0 mg N/L	1.912
107	2.0 mg N/L	1.883
108	2.0 mg N/L	1.933
109	2.0 mg N/L	1.955
110	2.0 mg N/L	1.963

Precision at Midscale

mean = 1.929
s = 0.023
%RSD = 1.18

--------- End of Report for Tray 92062507.RS -----------

1 (Ref: 92062503) 06/25/1992, 12:35 pm



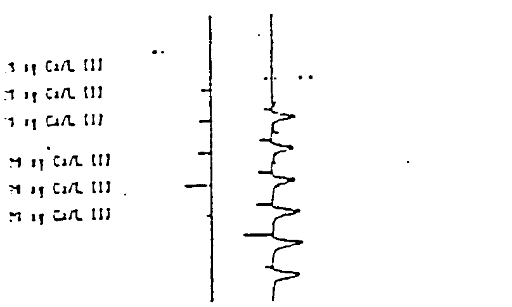
Cup	Sample ID	1 TKN mg N/L
101	1.0 mg N/L 4.8% H2SO4	0.966
102	1.0 mg N/L 4.4% H2SO4	0.960
103	1.0 mg N/L 4.0% H2SO4	0.894
104	1.0 mg N/L 4.8% H2SO4	0.975
105	1.0 mg N/L 4.4% H2SO4	0.925
106	1.0 mg N/L 4.0% H2SO4	0.888

Acid Effect

mean (4.8%) = 0.971
mean (4.4%) = 0.943
mean (4.0%) = 0.891

--------- End of Report for Tray 92062503.RS -----------

1 (Ref: 92062508) 06/25/1992, 01:14 pm



Cup	Sample ID	1 TKN mg N/L
101	20 mg Ca/L	-0.013<
102	20 mg Ca/L	0.005<
103	20 mg Ca/L	0.020<
104	100 mg Ca/L	0.052<
105	100 mg Ca/L	0.050<
106	100 mg Ca/L	0.060<

Calcium Interference Study

mean (20 mg/L) = 0.004
mean (100 mg/L) = 0.059
<MDL at 100 mg/L

--------- End of Report for Tray 92062508.RS -----------

17.4. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	90 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	45 s

Analyte data:

Peak Base Width: 31 s

% Width Tolerance: 100

Threshold: 25000

Inject to Peak Start: 42 s

Chemistry: Direct

Calibration Data:

Levels	1	2	3	4	5	6	7
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.25	0.10	0.00

Calibration Fit Type: 1st Order Polynomial

Weighting Method: None

Sampler Timing:

Min. Probe in Wash Period: 14 s

Probe in Sample Period: 20 s

Valve Timing:

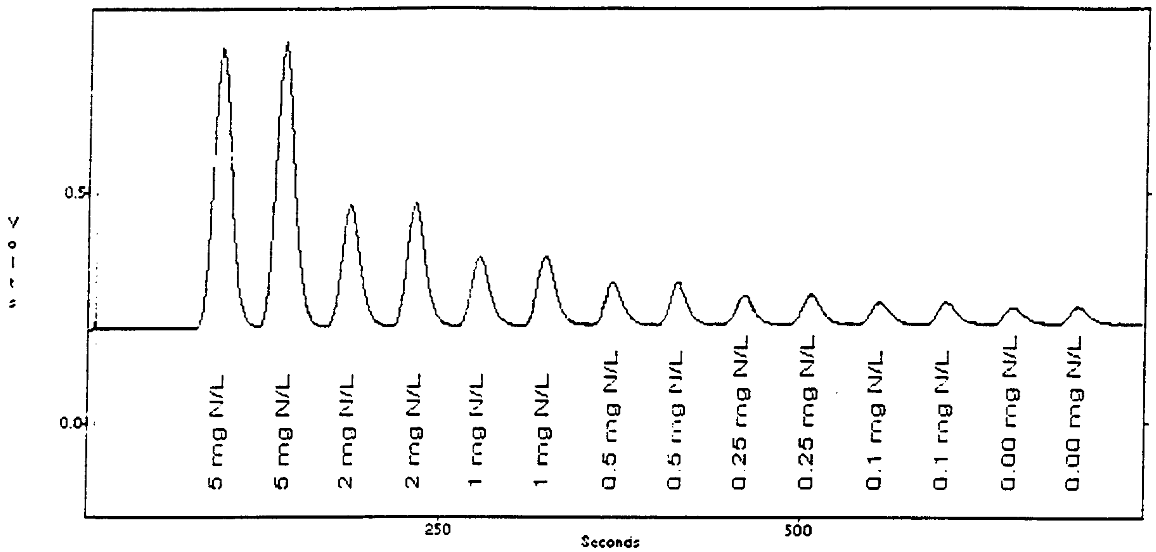
Load Period: 20 s

Inject Period: 25 s

Load Time: 0.0 s

17.5 QUIKCHEM 8000 SUPPORT DATA

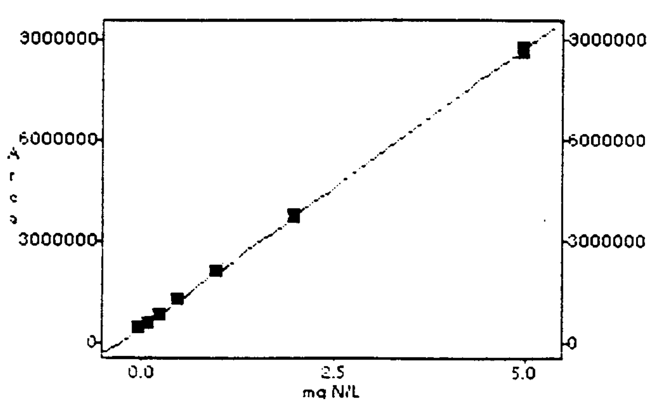
Figure 1. Calibration Graph and Statistics for Total Kjeldahl Nitrogen



ACQ. TIME: Aug 15, 1994 15:00:17
DATA FILENAME: C:\OMNION\DATA\1010762E\081594C1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1010762E\1010762E.met

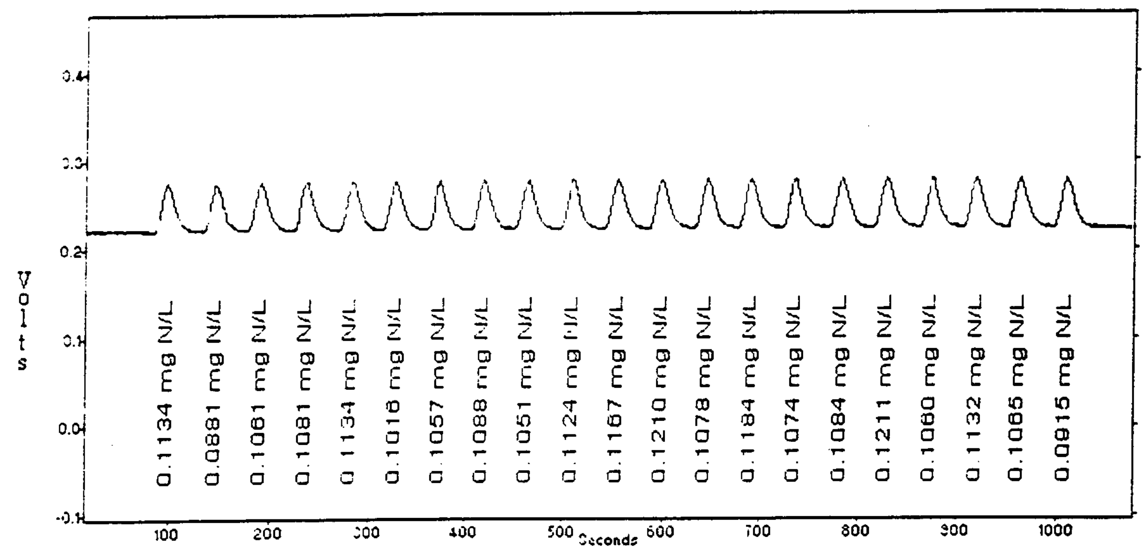
Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	8778128	5	5.000	8851078	8705178	1003166.9	1.2	0.0
2	3809886	2	2.002	3832259	3787513	31640.2	0.8	-0.1
3	2159521	1	1.006	2157189	2161852	3296.9	0.2	-0.6
4	1326319	0.5	0.5035	1335357	1317280	12782.8	1.0	-0.7
5	896683	0.25	0.2445	890758	902609	8380.0	0.9	2.2
6	643806	0.1	0.108	648879	638732	7174.7	1.1	8.0
7	498016	0	0	5071570	488876	12926.6	2.6	---



Scaling: None
Weighting: None
1st Order Poly
Conc = 6.032e-007Area-2.964e-001
$R^2 = 1.000$

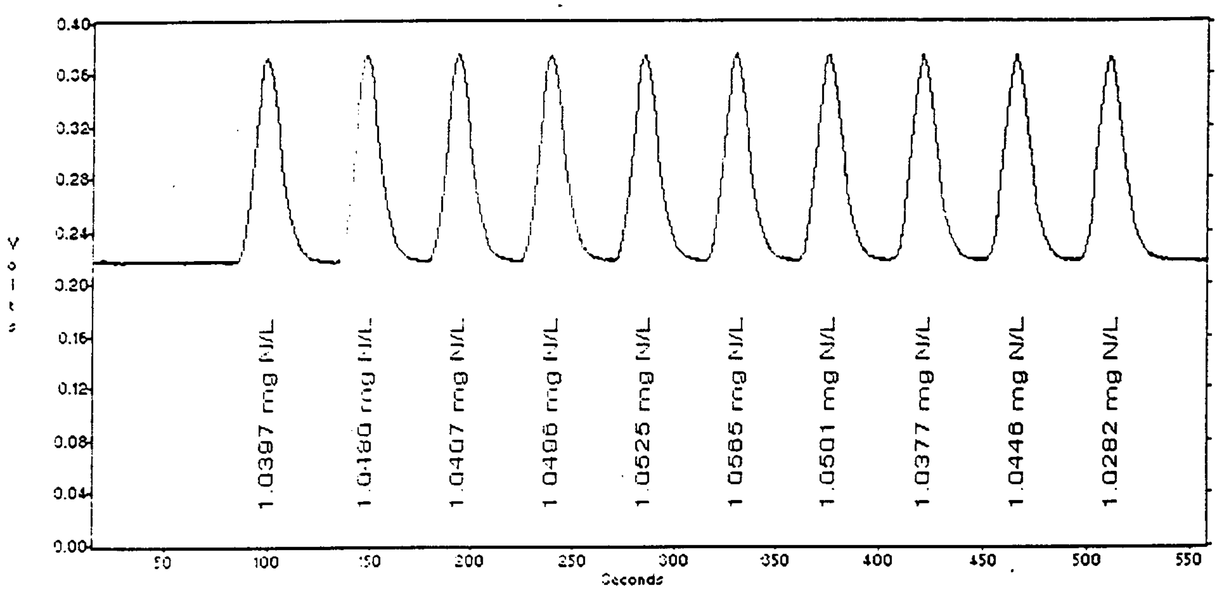
Figure 2. Method Detection Limit



MDL = 0.020 mg N/L

ACQ. TIME:	Aug 18, 1994 8:52:31
DATA FILENAME:	C:\OMNION\DATA\1010762E\081894M1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762E\1010762E.met

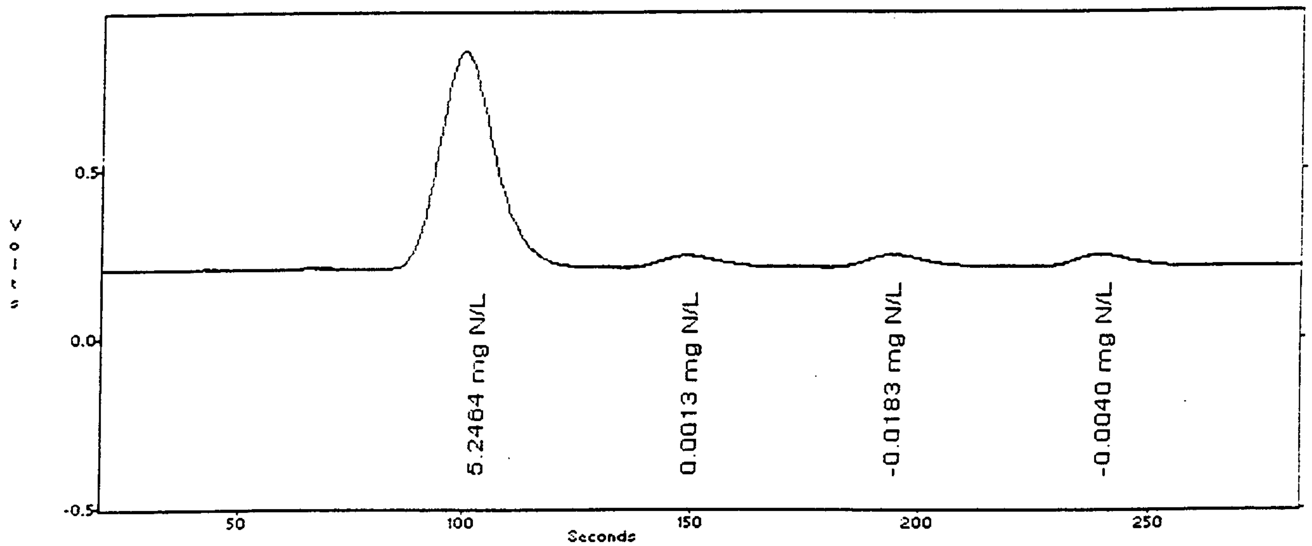
Figure 3. Precision



Precision = 0.796 % RSD

ACQ. TIME:	Aug 15, 1994 15:50:24
DATA FILENAME:	C:\OMNION\DATA\1010762E\081594P1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762E\1010762E.met

Figure 4. Carryover



Carryover passed

ACQ. TIME:	Aug 15, 1994 16:01:01
DATA FILENAME:	C:\OMNION\DATA\1010762E\081594R1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1010762E\1010762E.met

Technicon AutoAnalyzer II

INDUSTRIAL METHOD No. 334-74W/B+ RELEASED: JANUARY, 1976/REVISED: MARCH 1977

INDIVIDUAL/SIMULTANEOUS* DETERMINATION OF NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

RANGE: Nitrogen 1-50 mg/l; 20-1000 mg/l
Phosphorus 1-50 mg/l; 20-1000 mg/l

BD-20/BD-40 (DIALYZER)

GENERAL DESCRIPTION

NITROGEN

The determination of nitrogen is based on a colorimetric method in which an emerald-green color is formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite (chlorine source) in a buffered alkaline medium at a pH of 12.8-13.0. The ammonia-salicylate complex is read at 660 nm.

PHOSPHORUS

The determination of phosphorus is based on the colorimetric method in which a blue color is formed by the reaction of ortho phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH. The phosphomolybdenum complex is read at 660 nm.

The acid digest samples are prepared by digestion with the Technicon BD-40 or BD-20 Block Digestor. Refer to Manual No. TA4-0323-11 for sample preparation.

PERFORMANCE AT 40 SAMPLES PER HOUR

MANUALLY PREPARED STANDARDS

NITROGEN	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 1.00 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.6%	at 500 mg/l ±0.4%
Detection Limit	1.0 mg/l	20 mg/l

PHOSPHORUS	1-50 mg/l	20-1000 mg/l
Sensitivity	at 50 mg/l 0.20 absorbance unit	at 1000 mg/l 0.60 absorbance unit
Coefficient of Variation	at 25 mg/l ±0.5%	at 500 mg/l ±0.6%
Detection Limit	1.0 mg/l	20 mg/l

*See Operating Note 7.

TECHNICON INDUSTRIAL SYSTEMS / TARRYTOWN, N.Y. 10591
A DIVISION OF TECHNICON INSTRUMENTS CORPORATION

PRINTING CODE:
A/11/a/1
4732-1-6/R3-7-1.5

REAGENTS

Unless otherwise specified, all reagents should be of ACS quality or equivalent.

GENERAL REAGENTS

TRITON X-100 SOLUTION (50% in Methanol)

Triton X-100** (Technicon No. T21-0188)	50 ml
Methanol (CH_3OH)	50 ml

Preparation:
Add 50 ml of Triton X-100 to 50 ml of methanol and mix thoroughly.

SYSTEM WASH WATER SOLUTION (For System Shut-Down and Start-Up Only)

Triton X-100 Solution	1.0 ml
Distilled Water	1000 ml

Preparation:
Add 1.0 ml of Triton X-100 solution to one liter of distilled water and mix.

SAMPLER IV WASH RECEPTACLE SOLUTION

Distilled Water

Note: This reagent contains *no* wetting agent.

NITROGEN REAGENTS

STOCK SODIUM HYDROXIDE SOLUTION, 20%

Sodium Hydroxide Solution, 50% w/w	400 g
Distilled Water, q.s.	1000 ml

Preparation:
To 600 ml of distilled water, add 400 g of sodium hydroxide solution, 50% w/w. Cool to room temperature and dilute to one liter with distilled water.

STOCK SODIUM POTASSIUM TARTRATE SOLUTION, 20%

Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)	200 g
Distilled Water, q.s.	1000 ml

Preparation:
Dissolve 200 g of sodium potassium tartrate in about 600 ml of distilled water. Dilute to one liter with distilled water and mix thoroughly.

STOCK BUFFER SOLUTION 0.5*M*

Sodium Phosphate, Dibasic, crystal ($Na_2HPO_4 \cdot 7H_2O$	134 g
[Sodium Phosphate, Dibasic, anhydrous (Na_2HPO_4)]	[71 g]
Sodium Hydroxide Solution, 50% w/w	40 g
Distilled Water, q.s.	1000 ml

Preparation:
Dissolve 134 g of sodium phosphate, dibasic, crystal (or 71 g of sodium phosphate, dibasic, anhydrous) in about 800 ml of distilled water. Add 40 g of sodium hydroxide solution, 50% w/w, dilute to one liter with distilled water and mix thoroughly.

WORKING BUFFER SOLUTION

Stock Buffer Solution, 0.5*M*	200 ml
Stock Sodium Potassium Tartrate Solution, 20%	250 ml
Stock Sodium Hydroxide Solution, 20%	250 ml
Distilled Water, q.s.	1000 ml
Brij-35,*** 30% Solution (Technicon No. T21-0110)	1.0 ml

Preparation:
Combine the reagents in the stated order: add 250 ml of stock sodium potassium tartrate solution, 20%, to 200 ml of stock buffer solution, 0.5*M*, with swirling. Slowly, with swirling, add 250 ml of sodium hydroxide solution, 20%. Dilute to one liter with distilled water, add 1.0 ml of Brij-35, 30% solution, (20-25 drops) and mix thoroughly.

SULFURIC ACID/SODIUM CHLORIDE SOLUTION

Sulfuric Acid, 95-98% (H_2SO_4)	7.5 ml
Sodium Chloride (NaCl)	100 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% Solution	1.0 ml

Preparation:
Dissolve 100 g of sodium chloride in about 600 ml of distilled water. Add 7.5 ml of sulfuric acid and dilute to one liter with distilled water. Add 1.0 ml of Brij-35 (about 20 drops) and mix thoroughly.

SODIUM SALICYLATE/SODIUM NITROPRUSSIDE SOLUTION

Sodium Salicylate ($NaC_7H_5O_3$)	150 g
Sodium Nitroprusside [$Na_2Fe(CN)_5NO \cdot 2H_2O$]	0.30 g
Distilled Water, q.s.	1000 ml
Brij-35, 30% solution	1.0 ml

**Trademark of Rohm and Haas Company.

***Trademark of Atlas Chemical Industries, Inc.

Preparation:

Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 ml of distilled water. Filter through fast filter paper into a one liter volumetric flask and dilute to volume with distilled water. Add 1.0 ml of Brij-35 and mix thoroughly. Store in a light-resistant container.

SODIUM HYPOCHLORITE SOLUTION, 0.315%

Sodium Hypochlorite Solution, 5.25%	6.0 ml
Distilled Water, q.s.	100 ml
Brij-35, 30% Solution	0.1 ml

Preparation:

Dilute 6.0 ml of sodium hypochlorite solution to 100 ml with distilled water. Add 0.1 ml (2 drops) of Brij-35 and mix thoroughly. Prepare fresh daily. [Any commercial bleach solution (e.g. Clorox) containing 5.25% available chlorine is satisfactory.]

PHOSPHORUS REAGENTS

SULFURIC ACID SOLUTION, 4.0*N*

Sulfuric Acid, 95-98% (H_2SO_4)	111 ml
Distilled Water, q.s.	1000 ml
Triton X-100 Solution	1.0 ml

Preparation:

While swirling, cautiously add 111 ml of sulfuric acid to about 600 ml of distilled water. Cool to room temperature and dilute to one liter with distilled water. Add 1.0 ml of Triton X-100 solution and mix thoroughly.

SODIUM CHLORIDE SOLUTION, 0.25%

Sodium Chloride (NaCl)	2.5 g
Distilled Water, q.s.	1000 ml
Aerosol-22****	5.0

Preparation:

Dissolve 2.5 g of sodium chloride in about 600 ml of distilled water. Dilute to one liter with distilled water. Add 5.0 ml of Aerosol-22 and mix thoroughly.

MOLYBDATE/ANTIMONY SOLUTION

Ammonium Molybdate $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$	10.0 g
Antimony Potassium Tartrate $[K(SbO)C_4H_4O_6 \cdot 1/2H_2O]$	0.15 g
Sulfuric Acid, 95-98% (H_2SO_4)	60 ml
Distilled Water, q.s.	1000 ml

Preparation:

Dissolve 10.0 g of ammonium molybdate and 0.15 g of antimony potassium tartrate in about 800 ml of distilled water. While swirling, cautiously add 60 ml of sulfuric acid. Cool to room temperature, dilute to one liter with distilled water and mix thoroughly. Transfer to a light-resistant container. This solution is stable for about one month.

****Trademark of American Cyanamid Company.

ASCORBIC ACID SOLUTION, 1.0%

Ascorbic Acid ($C_6H_8O_6$) – OR – Araboascorbic Acid ($C_6H_8O_6$)	2.0 g
Distilled Water, q.s.	200 ml

Preparation:

Dissolve 2.0 g of ascorbic acid or araboascorbic acid in about 150 ml of distilled water. Dilute to 200 ml with distilled water and mix thoroughly. Transfer to a light-resistant container. If kept refrigerated and tightly stoppered when not in use, this solution is stable for at least two days.

OPERATING NOTES

1. Start-Up
   a. Check the level of all reagents to ensure an adequate supply.
   b. Excluding the salicylate and molybdate/antimony lines, place all reagent lines in their respective containers.
   c. When reagents have been pumping for at least five minutes, place the salicylate and molybdate/antimony lines in their respective containers and allow the system to equilibrate for 10 minutes.

   NOTE: If a precipitate appears after the addition of salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid is caused by a low pH. Before restarting the system, check the concentration of the sulfuric acid solution and/or the working buffer solution.

   d. To prevent precipitation of salicylic acid in the waste tray (which can clog the tray outlet), keep the nitrogen flowcell pump tube and the nitrogen colorimeter TO WASTE tube separate from all other lines or keep tap water flowing in the waste tray.

2. Shut-Down
   a. Remove the salicylate and molybdate/antimony lines from their containers and allow them to pump air. When the air bubbles enter the analytical system, place all reagent lines (excluding the Sampler IV Wash Receptacle Solution line) in the System Wash Water Solution.
   b. After 15 minutes, stop the proportioning pump and remove the platen.

3. **System Operation**
   a. Be sure the plastic cover of the analytical cartridge is in place when operating the system.
   b. At STD CAL settings of 6.00 or more, the system may be operated in the DAMP 1 position, if necessary.

4. **Manifold Connections**
   To avoid the possibility of airborne contamination, the air lines of the nitrogen channel should be attached to an air scrubber containing dilute sulfuric acid (10% v/v).

5. **Reagent Background Color**
   a. Place all lines in the system wash water container and start the proportioning pump. After making the necessary adjustments on the colorimeters set the STD CAL control of the nitrogen colorimeter to 1.00 and the STD CAL control of the phosphorus colorimeter to 2.90. Adjust the water baseline on both colorimeters to zero with the BLANK control.
   b. Following the start-up procedure, place all reagent lines in the proper order in their respective containers and allow the system to equilibrate.
   c. The reading of the reagents compared to distilled water should not be more than 14 units (0.140 absorbance) for the nitrogen channel and not more than 5 units (0.25 absorbance) for the phosphorus channel. If the absorbance of either channel is much higher than the above values, one or more of the reagents or the water used to make up the reagents is probably contaminated.

6. **Concentration Ranges**
   a. All concentration ranges refer to the concentration of components in the digestion tube after diluting to volume with distilled water.
   b. **Nitrogen Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the flowcell and the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure 1 and flow diagram).
      2. For any one manifold configuration, an approximate five-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 1.00 or higher.
   c. **Phosphorus Channel**
      1. Concentration ranges from 1-50 mg/l to 20-1000 mg/l can be accommodated by changing the size of the sample, resample and diluent lines as designated in the concentration ranges table (refer to Figure and flow diagram).
      2. For any one manifold configuration, an approximate three-fold change in concentration can be accommodated by use of the STD CAL control. The system is linear when operated at a STD CAL setting of 2.00 or higher.

7. **Manifold Configurations**
   a. **Individual Determination of N or P**
      When N or P is being determined individually, the PT fitting is omitted and the sample line is attached directly to the sample probe of the Sampler IV.
   b. **Simultaneous Determination of N and P**
      When N and P are being determined simultaneously, both initial sample lines are connected to a PT stream-splitter fitting which is in turn connected to the sample probe on the Sampler IV.

8. **Sample Probe and PT Stream-Splitter**
   Because stainless steel is susceptible to attack by sulfuric acid solutions, this method utilizes special Kel-F sample probe (Technicon No. 17 0745) and a special PT stream-splitter with platinum nipples (Technicon No. 116-B331).

9. **Phosphorus Channel (only)**
   a. **Cleansing Procedure**
      Before initially operating the system, the following procedure should be performed to cleanse the system. Once a week thereafter. this procedure should be repeated during system start-up.

      With the exception of the ascorbic acid and molybdate/antimony lines, place all phosphorus reagent lines into their respective containers. Start the proportioning pump and allow five minute pumping time. Place both the ascorbic acid and molybdate/antimony lines in sodium hydroxide solution, 20% for five minutes, then into hydrogen peroxide, 50% for five minutes. then into distilled water. After five minutes follow the start-up procedure (Operating Note 1) and allow the system to equilibrate.

b. **Conditioning Procedure**

After the initial cleansing of the system is performed, condition the phosphorus channel as described below. Once this channel has been conditioned, there is no need to repeat the procedure; only the cleansing procedure need be performed once each week during start-up.

Following the Start-Up procedure (Operating Note #1), place all reagent lines for phosphorus in their respective containers and allow the system to equilibrate. Place three sample cups containing midscale standard solution on the Sampler IV tray (with a stop-pin at the third cup) and start the sampler. Aspirate the set of standards three times, allowing five minutes of wash between each set. After the Recorder traces the last standard peak, wait ten minutes and adjust the baseline tracing to zero using the BASELINE control.

10. **Crude Protein Determination – AOAC**

When this methodology is utilized to assay acid digestates for the determination of Crude Protein in Feeds by the official AOAC procedure, the following hardware changes **must** be incorporated into the system:

a. **Sampler IV** — Sampler IV cam must be 40/hour with a sample-to-wash ratio of 2:1 (cam is included in the accessories and spares kit).

b. **Analytical Cartridge** — dilution loop pump tubes **must** be of the following size:

INITIAL SAMPLE DILUTION	
Sample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	1.20 ml/min (Yel/Yel)

RESAMPLE DILUTION	
Resample Line	0.16 ml/min (Orn/Yel)
H_2SO_4/NaCl Line	0.80 ml/min (Red/Red)

c. **Colorimeter** — must be equipped with 15 mm pathlength flowcell (1.5 or 2.0 mm ID).

INDIVIDUAL/SIMULTANEOUS
NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS
NITROGEN (RANGES: FROM 1-50 mg/l TO 20-1000 mg/l
MANIFOLD NO. 116-D531-01

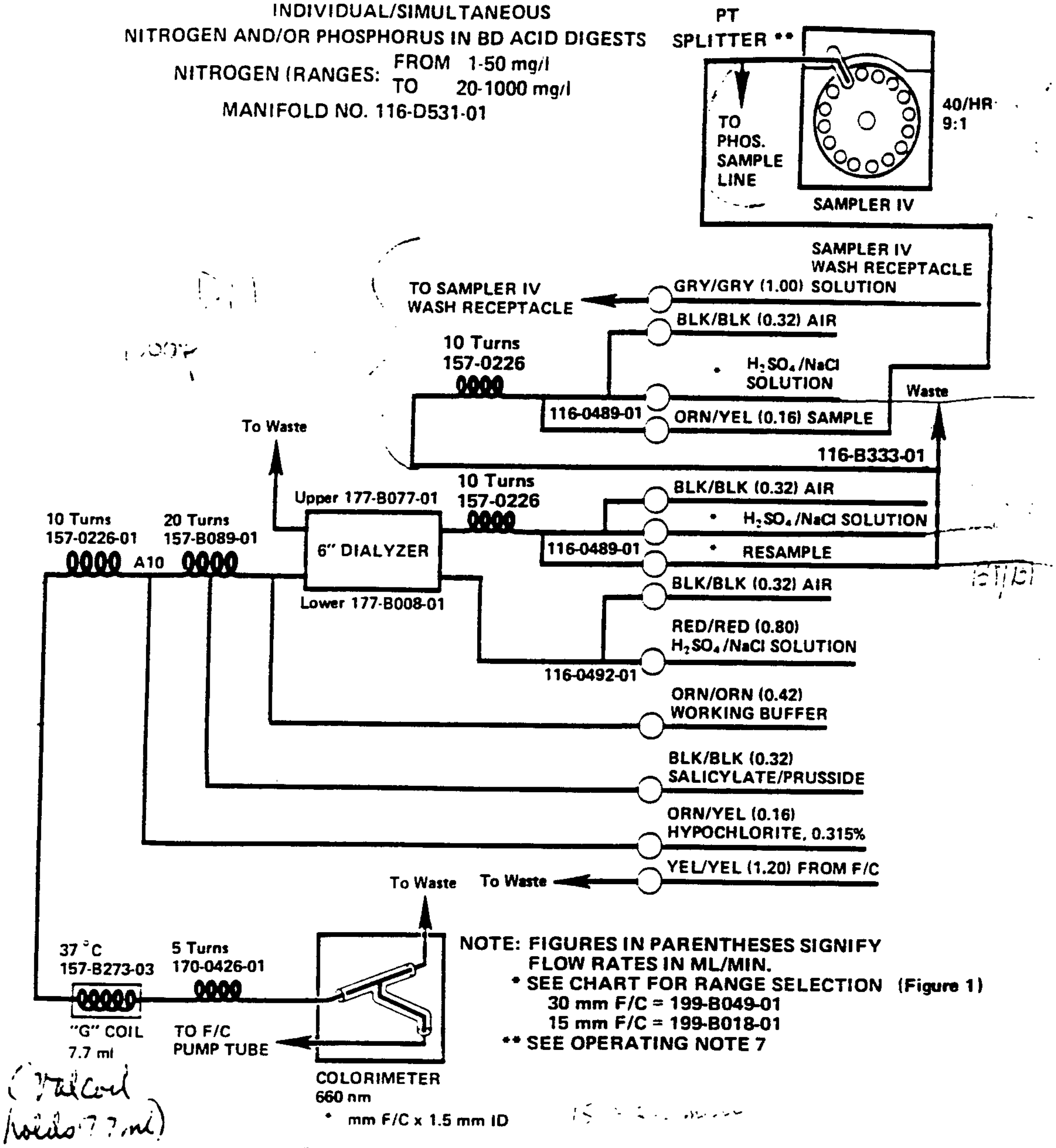


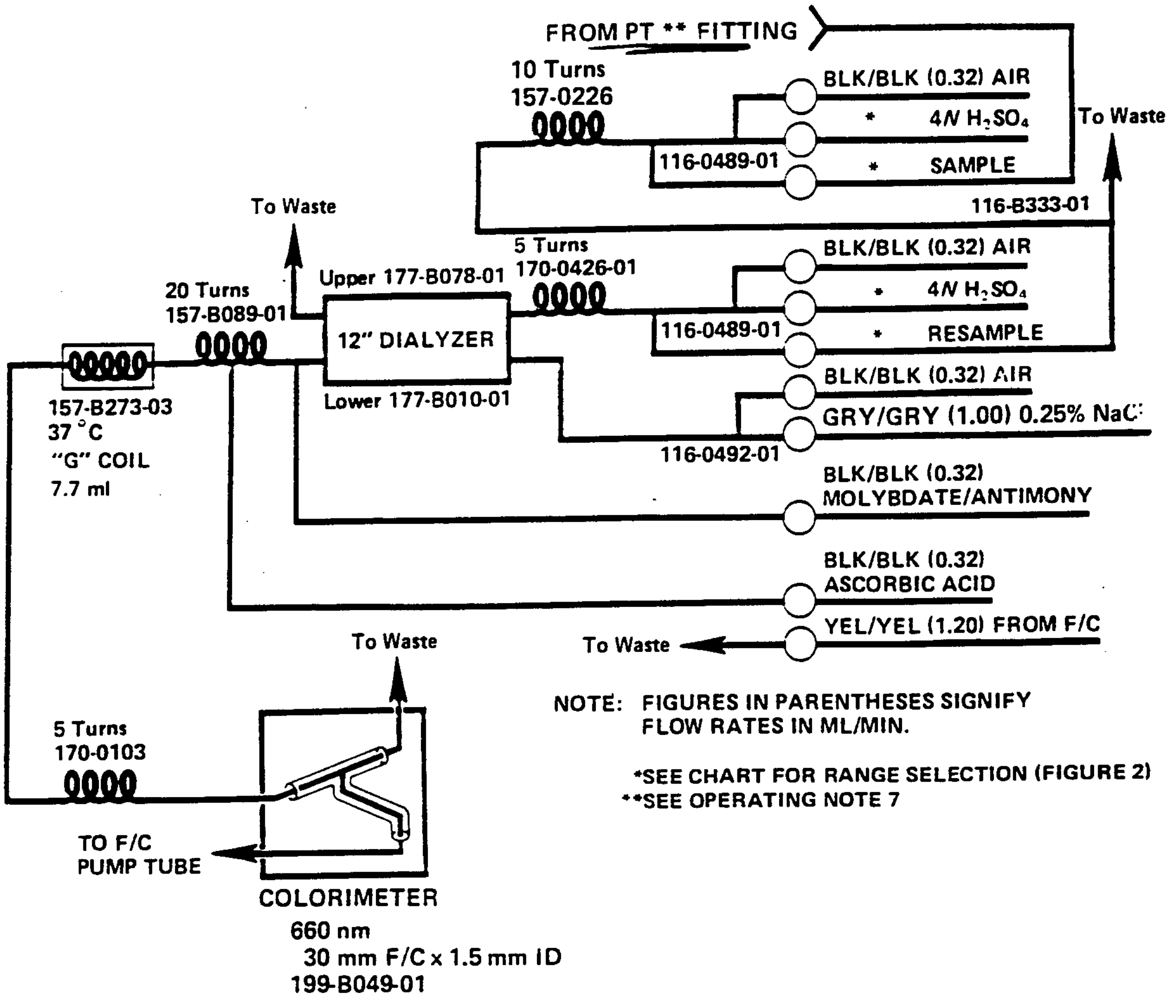
Figure 1. CONCENTRATION RANGES (NITROGEN)

DILUTION LOOPS				FLOWCELL PATH LENGTH (mm)	APPROX. STD CAL SETTING	RANGE PPM N (±10%)
INITIAL SAMPLE		RESAMPLE				
SAMPLE LINE	H_2SO_4/NaCl LINE	RESAMPLE LINE	H_2SO_4/NaCl LINE			
.16 (Orn/Yel)	1.20 Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	30	7.30	.7-35
					1.00	1.4-170
.16 (Orn/Yel)	1.20 Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30	1.2-60
					1.00	-300
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	30	7.30	2-100
					1.00	10-500
.16 (Orn/Yel)	1.20 (Yel/Yel)	.32 (Blk/Blk)	0.80 (Red/Red)	15	7.30	1.2-60
					1.00	6-300
.16 (Orn/Yel)	1.20 (Yel/Yel)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30	2.4-120
					1.00	12-600
.16 (Orn/Yel)	2.00 (Grn/Grn)	.16 (Orn/Yel)	1.00 (Gry/Gry)	15	7.30	4-200
					1.00	20-1000

INDIVIDUAL/SIMULTANEOUS NITROGEN AND/OR PHOSPHORUS IN BD ACID DIGESTS

PHOSPHORUS RANGES: FROM 1-50 mg/l TO 20-1000 mg/l

MANIFOLD NO. 116-D541-01



NOTE: FIGURES IN PARENTHESES SIGNIFY FLOW RATES IN ML/MIN.

*SEE CHART FOR RANGE SELECTION (FIGURE 2)

**SEE OPERATING NOTE 7

Figure 2. CONCENTRATION RANGES (PHOSPHORUS)

DILUTION LOOPS				APPROX. STD CAL SETTING	RANGE mg/l P (±10%)
INITIAL SAMPLE		RESAMPLE			
SAMPLE LINE	4N H_2SO_4 LINE	RESAMPLE LINE	4N H_2SO_4 LINE		
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.32 (Blk/Blk)	0.80 (Red/Red)	7.30 2.20	1-50 3-150
0.32 (Blk/Blk)	1.00 (Gry/Gry)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	2-100 6-300
0.16 (Orn/Yel)	1.60 (Blu/Blu)	0.16 (Orn/Yel)	1.00 (Gry/Gry)	7.30 2.20	6-300 20-1000

AutoAnalyzer Application

Industrial Method - #376-75W/B

DIGESTION AND SAMPLE PREPARATION FOR THE ANALYSIS OF TOTAL KJELDAHL NITROGEN AND/OR TOTAL PHOSPHORUS IN WATER SAMPLES USING THE BRAN + LUEBBE BD-40 BLOCK DIGESTOR

The following procedure is recommended for the analysis of nitrogen and/or phosphorus in water samples. Samples are digested using a Bran + Luebbe BD-40 Block Digestor and assayed using a Bran + Luebbe AutoAnalyzer II Continuous Flow Analytical System.

This procedure should be utilized in conjunction with the Operation Manual for the Block Digestor BD-20/40 (Bran + Luebbe Publication No. TA4-0323-11) and the methodology for Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests (Industrial Method No. 329-74W - Revised 11/78).

Introduction

When analyzing water samples with the BD-40, the water present in the digestion tubes must be evaporated before heating the tubes at a temperature that is high enough to affect digestion. When water is added to sulfuric acid, the boiling point of the resultant mixture is considerably lower than that of sulfuric acid alone. If this mixture is placed in the block at a temperature significantly higher than its boiling point, the tube contents will bump out resulting in loss of sample, contamination of adjacent tubes, and most importantly, possible bodily injury.

The automatic (temperature-programmed) mode of the BD-40 is utilized to first evaporate the water and then to raise and maintain the block temperature high enough to affect digestion. At the end of the programmed cycle, the unit automatically shuts down.

Since the concentration levels of N and P in the samples encountered are frequently very low, accuracy and precision can be insured only if good analytical technique is employed during all steps of the procedure -- from sample preparation to assay of the digested samples. Every precaution should be observed to avoid contamination of sample tubes, pipets, reagents, spatulas, etc. The use of de-ionized, distilled water or its equivalent is required throughout the procedure. Contaminated water is the most frequently encountered source of difficulty in running this procedure.

Concentration Ranges and Manifold Configuration

Samples

The choice of manifold configuration depends on the concentration of N and/or P in the sample.

Depending on the manifold configuration and STD CAL setting, the BD-40 related analytical cartridges can accommodate N and/or P in the ranges from 0.024 - 1.2 mg/l to 1.50 - 75 mg/l in the undigested sample.

The methodology (No. 329-74W) gives three configurations for the nitrogen and phosphorus cartridges and the concentration ranges for each of the configurations. Each configuration can accommodate approximately a five-fold change in concentration by varying the STD CAL control from 100 to 700. The range represents the detection limit (2% of full scale) and the full scale concentration for a particular STD CAL setting.

Samples containing higher levels of nitrogen or phosphorus should be diluted with distilled de-ionized water prior to digestion.

Referring back to Figures 1 and 2 of Method 329-74W: Because of the possibility of contamination, it is recommended that configuration #1 be used only for samples containing 15 mg N or P/1 or less; all samples greater than 15 mg/l should be assayed on configuration #2 or #3. The choice of cartridge configuration is best illustrated by example.

Consider a group of samples containing 1 - 5 mg N/l and 5 - 50 mg P/1. The configuration of choice for nitrogen would be #2 adjusted with the STD CAL control to 5 mg/l full scale deflection. This would yield peaks ranging from 20% to full scale. The choice for phosphorus would be configuration #3 adjusted for a full scale deflection of 50 mg/l. This would yield peaks ranging from 10% to full scale.

Occasionally, the range of N or P in the samples will not be as narrow as stated above; i.e., a few samples may be too low or too high for the range that was chosen for the majority of the samples. If so, the sample volume per tube may be adjusted up or down or the full scale deflection may be adjusted by means of the STD CAL.

Whenever possible, a cartridge configuration should be chosen such that adjustment of the STD CAL control will accommodate all the values encountered. Note that the STD CAL setting should not be changed while samples are being assayed; i.e., sample peaks can be compared to standard peaks only when both are run at the same STD CAL setting. If a STD CAL adjustment is anticipated, be sure to have on hand standards which will fall into the anticipated range.

Standards

The recommended standards for use with the BD-40 are aqueous solutions of ammonium sulfate [$(NH_4)_2SO_4$] for nitrogen and potassium dihydrogen phosphate (KH_2PO_4) for phosphorus. The volumes of standard solution to be used depend on the concentration range of the sample.

It is recommended that two standards for each parameter to be run: one at 30 - 40% of full scale and one at 70 - 80% of full scale.

Standards should be handled in exactly the same manner as samples; i.e., they should be pipetted into the BD tubes and carried through the entire digestion procedure.

A series of working standard solutions which can accommodate all the ranges of the method can be prepared utilizing the following stock solutions:

Stock Solution A (2.0 mg N/ml)

Ammonium Sulfate $(NH_4)_2SO_4$	0.9434 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.9434 g of ammonium sulfate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

Stock Solution B (2.0 mg P/ml)

Potassium Dihydrogen Phosphate (KH_2PO_4)	0.8788 g
Distilled Water, q.s.	100 ml

Preparation

Dissolve 0.8788 g of potassium dihydrogen phosphate in about 60 ml of distilled water. Dilute to 100 ml with distilled water and mix thoroughly.

The preparation of standards can be performed most readily if pipets ranging from 1 to 10 ml are available.

In Table #1, the extreme left column indicates the milliliters of stock solution to be diluted to one liter to obtain working standard solutions which will yield the concentration values in the second column. The same volumes diluted to 100 ml, or 10x those volumes diluted to one liter, will yield concentrations 10x these concentration values.

The preparation of standard solutions is best illustrated by an example. Using the example cited previously in the section on Samples, the N range was 1 - 5 mg/l and the P range was 5 - 50 mg/l.

For the N channel, an 80% of full scale standard would be 4.0 mg/l. From Table #1 4.0 mg/l can be obtained by using 8 ml per tube of a working standard solution containing 10 mg/l (Row 5, Column 8). Using 3 ml per tube of the same working solution will give 1.5 mg/l (Row 5, Column 3) or 30% of full scale.

Since 8 ml and 3 ml per tube were chosen for the nitrogen standards, the appropriate amount of P^- must also be present in those aliquots to accommodate the phosphorus channel. An 80% deflection for phosphorus corresponds to 40 mg P/l and a 30% deflection corresponds to 15 mg/l. Checking Column 8, 40 mg/l (10x chart value) can be obtained by using 8 ml stock solution B. The 30% value will automatically fall in range using the 3 ml aliquot. Hence, using 3 ml and 8 ml of a working standard solution prepared by diluting 5 ml of Stock Solution A plus 50 ml of Stock Solution B to one liter will yield the required N and P values.

The following general procedure may be used for preparation of standard solutions. Once the manifold configuration and concentration range have been chosen, choose a value (or 10x a chart value) from Table 1 which corresponds to an 80% deflection and which requires 5 ml per tube or more working standard solution. Using 5 ml or more for the 80% deflection insures that a smaller volume can be found on the chart which approximates the 30% deflection.

On a simultaneous system, either parameter may be determined first. Once the chart value has been chosen for one parameter, choose the value (or 10x a chart value) from the same column that most closely approximates an 80% deflection for the other parameter. Since the values are proportional to volume, the 30% values will automatically fall into range with each other.

Blanks

A duplicate blank determination (all reagents less sample) should be performed with each rack of samples by carrying the blank tubes through the entire digestion procedure.

Operating Procedure

Samples, Standards and Blanks

Samples and standards are pipetted directly into the digestion tubes. Samples should be pipetted in 20 ml aliquots. The amount of standard is determined by the level of the component(s) of interest.

Refer to Section II for guidelines on standard volumes and manifold configuration. Samples may be assayed singly or in duplicate, depending on workload. It is recommended that standards and blanks be assayed in duplicate.

While samples and standards are being prepared, pre-heat the block to 200°C by setting the HIGH TEMP dial to 200°C and depressing the MANUAL button.

Boiling Aids

Plain (not selenized) Hengar chips are utilized to promote smooth boiling during digestion. The addition of 2 - 3 chips per tube is recommended. The use of glass beads or perforated glass beads is not satisfactory to obtain smooth boiling.

Hengar chips are available from Arthur H. Thomas Company, Vine & Third Streets,

Philadelphia, PA. 19105. As an alternative, some users report a preference for acid-washed Chemware TFE (teflon) boiling stones. TFE boiling stones are available from Markson Science, Inc., Box 767, Delmar, California 92014.

Catalyst

Red mercuric oxide is recommended as a catalyst for the digestion of water samples. Because mercury can interfere in both chemistries, the amount added per tube is limited to an amount determined by the manifold configuration being utilized; i.e., the more the sample is diluted, the greater the amount of mercury that can be utilized. The amount of mercury recommended is 10 mg/tube. The mercury is most conveniently utilized as a solution of HgO in 10% sulfuric acid.

Preparation

Into a 100 ml volumetric flask, weigh 8.0 of red mercuric oxide. Add about 75 ml of 10% sulfuric acid and stir until dissolved. Dilute to 100 ml with 10% sulfuric acid and mix thoroughly.

Digestion (Salt/Acid/Catalyst) Mixture

To insure uniform blank values from tube to tube, it is recommended that potassium sulfate, sulfuric acid and catalyst be added to each tube as a single mixture rather than as separate components. The procedure below may be used to prepare the digestion mixture.

Prepare and store the mixture in a stoppered container to minimize the possibility of airborne contamination. The mixture may be prepared in as large a quantity as is practical to handle and store.

Preparation

Carefully add 200 ml of concentrated sulfuric acid to 700 ml of de-ionized distilled water. Dissolve 133 g of potassium sulfate into this mixture, add 25 ml of mercuric sulfate catalyst solution and dilute to one liter with de-ionized distilled water.

For most applications, 5 ml of digestion mix per tube is satisfactory.

The utilization of a plunger-type repetitive dispensing device offers a rapid, convenient method of adding the digestion mixture to the tubes. When a plunger-type dispenser is utilized, the dispenser must be broken down, cleaned with water and air dried every three days. Failure to clean the plunger every three days can cause the plunger to freeze in the barrel of the dispenser due to crystallization of potassium sulfate.

Digestion

After samples, standards and reagents have been added to the digestion tubes, the water must be evaporated before high temperature digestion can be performed.

Place the loaded rack into the pre-heated block (200°C) and attach the end plates to the rack. The plates should remain in place until the rack is removed from the block. End plates promote water evaporation during low temperature operation and insure proper refluxing of the acid during high temperature digestion.

When the loaded rack is placed in the block, set the programmer as follows and then depress the AUTO button:

Total Cycle Time:	2 1/2 hours
High Temp °C:	380 °C
Low Temp Time:	1 hour
Low Temp °C:	200 °C

Under these conditions, the until will operate for a total cycle of 2 1/2 hours: 1 hour at 200 °C, about 1 hour to heat up to 380 °C and about 1/2 hour at 380 °C. At the end of 2 1/2 hours, the until will automatically shut down.

Cooling and Dilution

At the end of the program cycle (2 1/2 hours), remove the rack from the block, place it on an asbestos pad or in the cooling rack and remove the metal end plates. Allow the tubes to cool for about 5 minutes before diluting with 20 ml of de-ionized distilled water. Tubes are cool enough to dilute when the white acid fumes have dissipated and the upper half of the tube is cool enough to handle comfortably. The tubes should not be allowed to cool to the point of K_2SO_4 precipitation.

With the aid of a vortex type tube mixer, add to each tube, while swirling, 20 ml of deionized distilled water using a repetitive pipetter. Add the water in one continuous portion at a moderate rate and angle the tube away from the face. Allow the tube contents to mix thoroughly.

The tube contents should be at room temperature before analyzing. The tubes may be cooled rapidly by placing the entire rack into a sink partially filled with cold water.

Analysis

After cooling to room temperature, the digests may be analyzed using Bran + Luebbe Methodology No. 329-74W -- Individual/Simultaneous Determination of Nitrogen and/or Phosphorus in BD Acid Digests, Revised 11/78.

Transfer to glass sample cups, which have been previously acid washed and dried.

Before analyzing the entire set of samples, standards and blanks, run a few standard cups through the system and with the STD CAL control, adjust the standard peaks to the proper chart reading.

TABLE 1: CONCENTRATION OF STANDARD IN DIGESTS

*Ml Stock Solution A or B	Working Standard Conc. Mg N or P/L	Ml Working Standard Solution Per Tube and Resulting Digest Concentration in Mg N or P/L									
		1	2	3	4	5	6	7	8	9	10
1	2	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
2	4	.2	.4	.6	.8	1.	1.2	1.4	1.6	1.8	2
3	6	.3	.6	.9	1.2	1.5	1.8	2.1	2.4	2.7	3
4	8	.4	.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4
5	10	.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5
6	12	.6	1.2	1.8	2.4	3.0	3.6	4.2	4.8	5.4	6
7	14	.7	1.4	2.1	2.8	3.5	4.2	4.9	5.6	6.3	7
8	16	.8	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8
9	18	.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9
10	20	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10

*Dilute to 1000 ml to get chart values

Dilute to 100 ml to get 10X chart values

Stock Solution A = Nitrogen

Stock Solution B = Phosphorus

APPENDIX A-11

Procedure for Nitrate and Nitrite Nitrogen: Method 353 Series

1.0 PURPOSE

This procedure provides a method for the determination of nitrate and nitrite in drinking, ground, and surface water, and domestic and industrial wastes.

2.0 SCOPE

2.1 This method covers the determination of nitrate and nitrite in drinking, ground, and surface waters, and domestic and industrial wastes.

2.2 The method is based on reactions that are specific for the nitrate and nitrite (NO_3^- and NO_2^-) ions.

2.3 The applicable range is 0.2 to 20.0 mg N/L.

3.0 SUMMARY

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone can be determined by removing the cadmium column. Nitrate may be determined by difference.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, "Nitrogen, Nitrate-Nitrite, Method 353.2 (Colorimetric, Automated, Cadmium Reduction)."

4.2 *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments*. Book 5. Chapter A1. U.S Department of the Interior, U.S. Geological Survey.

4.3 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-107-04-1-A, "Nitrate/Nitrite, Nitrite in Surface Water, Wastewater."

4.4 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter (preferred - 1 mL of 1N H_2SO_4 per 100 mL) and stored at 4°C. Acid preserved samples have a holding time of 28 days.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is above the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Residual chlorine can interfere by oxidizing the cadmium column.

6.2.2.2 Low results may be obtained for samples that contain high concentrations of iron, copper of other metals. In this method, EDTA is added to the buffer to reduce this interference.

6.2.2.3 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference may be eliminated by extracting such samples with an organic solvent prior to analysis.

6.2.2.4 Sample color and turbidity may interfere. Turbidity can be removed by filtration through a 0.45 um pore diameter membrane filter prior to analysis. Sample color may be corrected by running the samples through the manifold without color formation (Sulfanilamide color reagent, reagent 3). The nitrate concentration is determined by subtracting the value obtained without color formation from the value obtained with color formation.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimeter detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Cadmium Granules Column

6.3.4.1.1 Cadmium Preparation: Place **10-20 g of coarse cadmium granules** (0.3 - 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with **50 mL of acetone**, then **water**, then two **50 mL portions of 1 N hydrochloric acid** (reagent 4). Rinse several times with water. **Cadmium is toxic and carcinogenic. Wear gloves.**

6.3.4.1.2 Copperization: Add a **100 mL** portion of **2% copper sulfate solution** (reagent 5) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh **2% copper sulfate solution** (reagent 5). Continue this process until the blue aqueous **copper** color persists. Decant and wash with at least **five portions of ammonium chloride buffer solution** (reagent 2) to remove colloidal copper. The cadmium should be black or dark gray. The copperized granules may be **stored** in a **stoppered bottle under ammonium chloride buffer** (reagent 2).

6.3.4.1.3 Packing the Column

6.3.4.1.3.1 The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that both hands are free. Unscrew one of the colored fittings from an end of the column. Pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting higher than the open end of the column and completely fill the column, attached fittings, and tubing with ammonium chloride buffer (reagent 2).

6.3.4.1.3.2 Scoop up the prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with water.

6.3.4.1.3.3 If air remains in the column or is introduced accidentally, connect the column into the manifold at the two state switching valve, pump ammonium chloride buffer (reagent 2) through the column with the pump on maximum, and tap firmly with a screwdriver handle, working up the column until all air is removed.

6.3.4.1.4 Cadmium Granules Column Instillation To Manifold

6.3.4.1.4.1 Before inserting the column, pump all reagents into the manifold.

6.3.4.1.4.2 Turn the pump off and immediately connect both column tubes to the two state switching valve used to place the column in line with the manifold. Do not let air enter the column.

6.3.4.1.4.3 Return the pump to normal speed. The direction of reagent flow through the column is not relevant.

6.3.4.2 Cadmium Wire Column

6.3.4.2.1 Join **two glass tubes, 122 cm x 1.5 mm each**, and bend into a "**U**" shape about **4 cm** apart. Secure the tubes on a **122 cm x 10 cm board** to prevent breaking. Let the open ends of the tubes **extend over the board about 5 cm** to make connections.

6.3.4.2.2 Cut **two 127 cm lengths of 0.050 inch diameter cadmium alloy wire** (95% cadmium, 5% silver).

6.3.4.2.3 Cadmium Wire Preparation: Wash wire with **acetone** to remove oil and grease, then **water**, then with **1 N hydrochloric acid** (reagent 4) to remove oxides. Rinse several times with **water**. **CAUTION: Collect and store all waste cadmium. Cadmium is toxic and carcinogenic. Wear gloves.**

6.3.4.2.4 Place the two lengths of cadmium wire into the two legs of the column using pliers. Push only about **5 to 6 mm** of wire at a time into the tube to avoid bending and kinking the wire. Push each wire down to the bend in the column as far as it will go. Connect the column to the **two state switching valve** on the manifold using short lengths of Tygon tubing and available fittings. Care should be taken to **minimize any dead volume**.

6.3.4.2.5 Copperization: Pump **2% copper sulfate solution** (reagent 5) through the column until the wire has a metallic appearance. Pump **ammonium chloride solution** (reagent 2) through the column for three to four minutes to remove colloidal copper. **Store** the column filled with **ammonium chloride solution** (reagent 2).

6.3.4.2.6 Cadmium Wire Column Instillation To Manifold

6.3.4.2.6.1 Before inserting the column, pump all reagents into manifold.

6.3.4.2.6.2 Turn the pump off and immediately connect both column tubes to the two state switching valve used to place the column in-line with the manifold.

6.3.4.2.6.3 Set the pump to normal speed.

6.3.4.2.6.4 The direction of reagent flow through the column is not relevant.

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

6.4 Reagents and Standards

6.4.1 Preparation of Reagents

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas all solutions except the standards with helium. Bubble helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute.

Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. 15 N Sodium Hydroxide**

Add **150 g NaOH pellets** very slowly to **250 mL or g of water** or add **300 g 50% NaOH solution** very slowly to **100 mL or g of water**. **CAUTION**: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

6.4.1.2 **Reagent 2. Ammonium Chloride buffer, pH 8.5**

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA \cdot 2H_2O$) in about **800 mL water**. Dilute to the mark and shake or stir to mix. Adjust the pH to **8.5** with **15 N sodium hydroxide solution** (reagent 1).

By weight: To a tared **1L** container, add **85.0 g ammonium chloride** (NH_4Cl), **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA \cdot 2H_2O$) and **938 g water**. Shake or stir until dissolved. Then adjust the pH to **8.5** with **15 N sodium hydroxide solution** (reagent 1).

6.4.1.3 **Reagent 3. Sulfanilamide color reagent**

By Volume: To a **1 L** volumetric flask add about **600 mL water**. Then add **100 mL of 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake to wet, and stir with a stir bar for 30 minutes to dissolve. Dilute to the mark, invert or stir to mix. Store in a dark bottle.

By weight: To a tared, dark **1 L** container add **876 g water, 170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake to wet, and stir with stir bar for 30 minutes until dissolved. Store in a dark bottle.

6.4.1.4 **Reagent 4. 1 N Hydrochloric Acid (HCl)**

By Volume: In a **100 mL** container, add **8 mL concentrated HCl** to **92 mL water**. Stir or shake to mix.

By weight: To a **100 mL** container, add **92 g water** then add **9.6 g concentrated HCl**. Stir or shake to mix.

6.4.1.5 **Reagent 5. 2% Copper Sulfate Solution**

By Volume: In a **1 L** volumetric flask, dissolve **20 g copper sulfate pentahydrate** ($CuSO_4 \cdot 5H_2O$) in about **800 mL water**. Dilute to mark with **water**. Invert to mix thoroughly.

By Weight: To a **1 L** container, add **20 g copper sulfate pentahydrate** ($CuSO_4 \cdot 5H_2O$) to **991 g water**. Stir or shake to dissolve.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneous for PO_4-P, NH_3-N and NO_2-N + NO_3-N. Also included is the preparation of a NO_2-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO_4^{3-}**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) for one hour at 105°C. In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH_3**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO_3^-**

In a **1 L** volumetric flask dissolve **7.220 g potassium nitrate** (KNO_3) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO_2^-**

In a **1 L** volumetric flask dissolve **4.93 g sodium nitrate** ($NaNO_2$) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO_4-P, NH_3-N and NO_3-N**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with **water** and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO_2^-**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **20 mL Stock Nitrate Standard** (standard 4). Dilute to mark with **water** and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO_4^{3-}, 31.06 mg N/L as NH_4, and 27.11 mg N/L as NO_3^-.**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the E M Science **1000 mg/L Phosphate Standard Solution** (326.1 mg P/L), **20 mL** of the E M Science **1000 mg/L Ammonia Standard Solution** (776.5 mg N/L), and **60 mL** of the E M Science **1000 mg/L Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water.**

	Calibration Standards Concentration mg/L	Prepared From Concentration mg/L	Prepared From Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H_2SO_4 added per 100 mL, add **5 mL** of **1N H_2SO_4** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO_2^-**

In a **500 mL** volumetric flask add about **300 mL water.** Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water,** add **5 mL** of **1N H_2SO_4** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO_4^{3-}, 1.55 mg N/L as NH_3, and 1.36 mg N/L as NO_3^-.**

In a **1 L** volumetric flask add about **700 mL water.** Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water,** add **10 mL** of **1N H_2SO_4** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, a cadmium reduction column efficiency check standard, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above four QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.3.

7.1.4 Enter data system parameters as in section 9.1 or 9.2.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 3).

7.1.10 At end of run, turn the two state switching valve to isolate the cadmium reduction column. Remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual, page 43, Task 11 - Creating a Custom Report)

7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.

7.2.5 Report results in mg NO_3-N/L.

8.0 <u>SAFETY</u>

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these

reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters - Cadmium Granules Reduction Column

Method Filename:	PANHANOA.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name:	Nitrate (NO_3)-N
Concentration Units:	mg NO_3-N/L
Chemistry:	Direct
Inject to Peak Start (s):	22.0
Peak Base Width (s):	29.000
% Width Tolerance:	100.000
Threshold:	4100.000
Autodilution Trigger:	Off
QuickChem Method:	10-107-04-1-A

Calibration Data:

Levels: (mg NO_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000

Calibration Rep Handling:	Average
Calibration Fit Type:	1st Order Poly
Force through Zero:	No
Weighing Method:	None

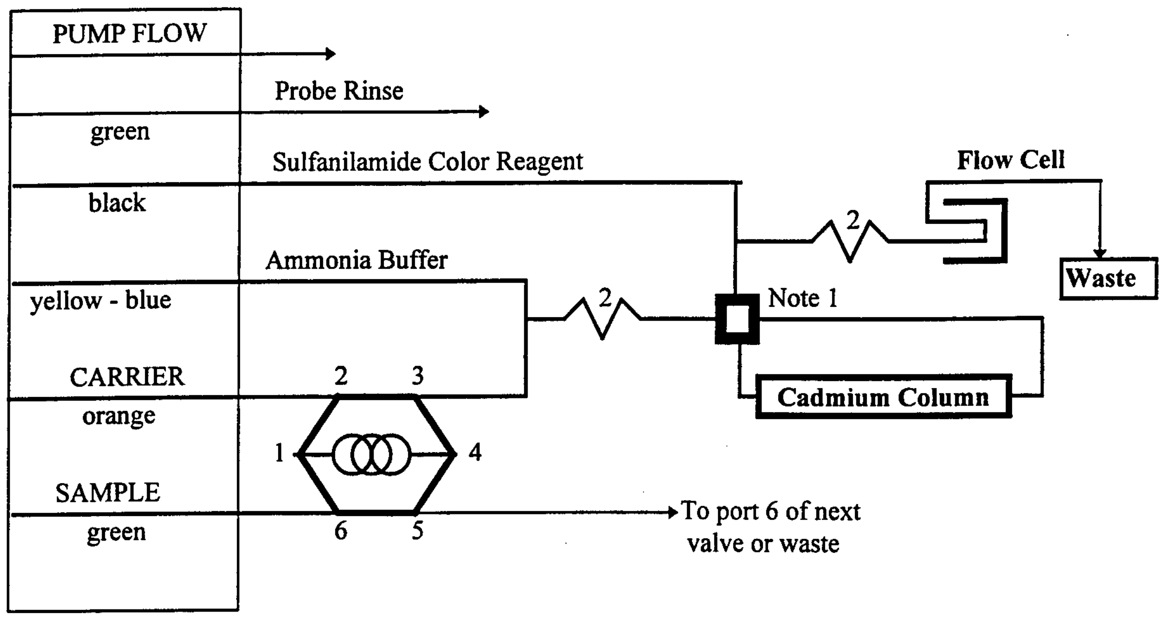
Concentration Scaling:	None
Sampler Timing:	
Method Cycle Period:	50.0
Min. Probe in Wash Period:	9.0
Probe in Sample Period:	25.0
Valve Timing:	
Method Cycle Period:	50.0
Sample Reaches 1st Valve:	18.0
Valve:	On
Load Time:	0.0
Load period	20.0
Inject Period:	30.0
Sample Loop:	Microloop

9.2 Data System Parameters - Cadmium Wire Reduction Column

Method Filename:	PANHANOW.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L
Analyte Data:	
Analyte Name:	Nitrate (NO_3)-N
Concentration Units:	mg NO_3-N/L
Chemistry:	Direct
Inject to Peak Start (s):	50.5
Peak Base Width (s):	29.000
% Width Tolerance:	100.000

Threshold:	4100.000		
Autodilution Trigger:	Off		
QuickChem Method:	10-107-04-1-A		
Calibration Data:			
Levels: (mg NO_3-N/L)	1: 20.000	2: 10.000	3: 4.000
	5: 1.000	6: 0.100	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		
Sampler Timing:			
Method Cycle Period:	70.0		
Min. Probe in Wash Period:	9.0		
Probe in Sample Period:	30.0		
Valve Timing:			
Method Cycle Period:	70.0		
Sample Reaches 1st Valve:	18.0		
Valve:	On		
Load Time:	0.0		
Load period	25.0		
Inject Period:	45.0		
Sample Loop:	Microloop		

9.3 Nitrate Manifold Diagram



Sample Loop = Microloop Interference Filter = 520 nm

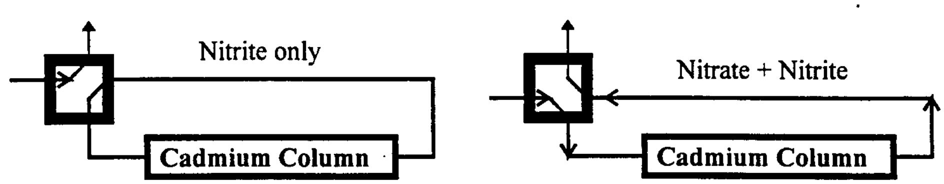
Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**.

2 is **70 cm** of tubing on a **4.5 cm** coil support.

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

APPENDIX A-12

Procedure for Orthophosphate: Method AP-0060

1.0 PURPOSE

This procedure provides a method for the determination of orthophosphate in drinking, ground, and surface waters, and domestic and industrial wastes.

2.0 SCOPE

2.1 This method covers the determination of orthophosphate in drinking, ground, and surface waters, and domestic and industrial wastes.

2.2 This method is based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

2.3 The applicable range is 0.02 to 4.00 mg P/L.

3.0 SUMMARY

3.1 Only orthophosphate forms a blue color in this test. Polyphosphates and organic phosphorus compounds are not recovered. The sulfuric acid in the molybdate reagent does not have enough time with polyphosphates to hydrolyze them.

3.2 The orthophosphate reacts with ammonium molybdate and potassium tartrate under acidic conditions to form an antimony-phosphomolybdate complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

4.0 REFERENCES

4.1 U.S. Environmental Protection Agency, *Methods for the Determination of Inorganic Substances in Environmental Samples*, EPA-600/R-93/100, August 1993, "Phosphorus, All Forms, Method 365.1 (Colorimetric, Automated, Ascorbic Acid)."

4.2 *Methods for Determination of Inorganic Substances in Water and Fluvial Sediments*. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

4.3 *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, p. 4 - 116, Method 4500-P F (1992).

4.4 *Guideline and Format for EMSL-Cincinnati Methods*. EPA-600/8-83-020, August 1983.

4.5 Lachat Instruments, *QuickChem Automated Ion Analyzer Methods Manual*, QuickChem Method 10-115-01-1-A, "Determination Of Orthophosphate In Waters By Flow Injection Analysis Colorimetry."

4.6 Lachat Instruments, *QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual.*

5.0 RESPONSIBILITIES

5.1 It is the responsibility of the laboratory manager to ensure that this procedure is followed.

5.2 It is the responsibility of the team leader to review the results of the procedure.

5.3 It is the responsibility of the Analysts to follow this procedure, evaluate data, and to report any abnormal results or unusual occurrences to the team leader.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned (use phosphate-free detergents), acid rinsed with 1:1 HCl, then rinsed with reagent water. The volume collected should be sufficient to ensure a representative sample and allow for quality control analysis (at least 100 mL).

6.1.2 The USEPA recommends that samples be stored at 4° C with a maximum holding time of 48 hours, and that samples for dissolved phosphorus be filtered immediately upon collection.

6.1.3 If samples of high (pH > 8) are suspected add 1 drop of phenolphthalein indicator to a 50 mL aliquot of sample. If a red color develops, add 11 N sulfuric acid (310 mL concentrated H_2SO_4 /L) drop-wise to just discharge the color. If samples have been acidified at collection, match the acid concentration in each calibration standard.

6.2 Limitations and Actions

6.2.1 If the analyte concentration is not within the analytical range of the calibration curve, the sample must be diluted to bring the analyte concentration within range.

6.2.2 Interferences

6.2.2.1 Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

6.2.2.2 Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

6.2.2.3 For dissolved orthophosphate, sample turbidity must be removed by filtration prior to analysis. Sample color that absorbs at 880 nm will also interfere. When in doubt about background absorbance, the background concentration should be determined.

6.3 Apparatus/Equipment

6.3.1 Balance – analytical, capable of accurately weighing to the nearest 0.0001 g.

6.3.2 Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3.3 Flow injection analysis equipment (Lachat model 8000) designed to deliver and react samples and reagents in the required order and ratios.

6.3.3.1 Autosampler

6.3.3.2 Multichannel proportioning pump

6.3.3.3 Reaction unit or manifold

6.3.3.4 Colorimetric detector

6.3.3.5 Data system

6.3.4 Special Apparatus

6.3.4.1 Heating Unit

6.3.5 Syringe filters - Titan nylon 25-mm syringe filters - 0.45 micron. SRI Catalog number 44525-NN or equivalent.

6.3.6 Syringes - 10 cc syringe with Luer Lok, B-D Part 309604 or equivalent. (Smaller volumes are acceptable)

6.4 Reagents and Standards

6.4.1 Preparation of Reagents

Use deionized water (10 megohm) for all solutions.

Degassing with helium: To prevent bubble formation, degas the carrier solution and other reagents as noted with helium. Bubble Helium through a degassing tube (Lachat Part 50100) through the solution for at least one minute. Refrigerate all solutions and standards.

6.4.1.1 **Reagent 1. Stock Ammonium Molybdate Solution**

By Volume: In a **1 L** volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ in approximately **800 mL water**. Dilute to mark with **water** and stir for **two hours**. Store in plastic and refrigerate.

By Weight: To a tared **1 L** container add **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24} \cdot 4H_2O]$ and **983 g water**. Stir for **two hours**. Store in plastic and refrigerate.

6.4.1.2 **Reagent 2. Stock Antimony Potassium Tartrate Solution**

By Volume: In a **1 L** volumetric flask dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$) in approximately **800 mL of water**. Dilute to mark with **water** and mix. Store in a dark bottle and refrigerate.

By Weight: To a **1 L dark** tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$) and **995 g water**. Mix. Store in a dark bottle and refrigerate.

6.4.1.3 **Reagent 3. Molybdate Color Reagent**

By Volume: To a **1 L** volumetric flask add about **500 mL water**, then add **35.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 mL Stock Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark with **water** and mix. Degas with helium and refrigerate.

By Weight: To a tared **1 L** container add **680 g water**, then **64.4 g concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 g Stock Antimony Potassium Tartrate Solution** (Reagent 2) and **213 g Stock Ammonium Molybdate Solution** (Reagent 1). Mix and degas with helium. Refrigerate.

6.4.1.4 **Reagent 4. Ascorbic Acid Reducing Solution, 0.33 M**

By Volume: In a **1 L** volumetric flask dissolve **60.0 g granular ascorbic acid** in about **700 mL water.** Dilute to the mark with **water**, mix and degas. After degassing add **1.0 g dodecyl sodium sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Refrigerate. Discard if solution becomes yellow.

By Weight: To a tared **1 L** container add **60.0 g granular ascorbic acid** and **975 g water**. Stir until dissolved then degas. After degassing add **1.0 g dodecyl sodium sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Refrigerate. Discard if solution becomes yellow.

6.4.1.5 **Reagent 5. Sodium Hydroxide - EDTA Rinse**

Dissolve **65 g sodium hydroxide** (NaOH) and **6 g tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in **1.0 L** or **1.0 kg water**.

6.4.2 Preparation of Standards

Note: Following are standards preparations for running 3 channels simultaneous for PO_4-P, NH_3-N and NO_2-N + NO_3-N. Also included is the preparation of a NO_2-N standard which is used to assess the cadmium reduction column's efficiency.

6.4.2.1 **Standard 1. Stock Orthophosphate Standard - 1000 mg P/L as PO_4^{3-}**

Dry **primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) for one hour at 105°C. In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.2 **Standard 2. Stock Ammonia Standard - 1000 mg N/L as NH_3**

Dry **ammonium chloride** (NH_4Cl) for two hours at 105°C. In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) in about **800 mL water**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.3 **Standard 3. Stock Nitrate Standard - 1000 mg N/L as NO_3^-**

In a **1 L** volumetric flask dissolve **7.220 g potassium nitrate** (KNO_3) in about **600 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.4 **Standard 4. Stock Nitrite Standard - 1000 mg N/L as NO_2^-**

In a **1 L** volumetric flask dissolve **4.93 g sodium nitrate** ($NaNO_2$) in about **800 mL water**. Add **2 mL chloroform**. Dilute to mark with **water** and mix. Refrigerate. This solution is stable for six months.

6.4.2.5 **Standard 5. Working Standard - 50 mg/L PO_4-P, NH_3-N and NO_3-N**

In a **1 L** volumetric flask add about **600 mL water**. Pipette **50 mL** from each of the **Stock Orthophosphate Standard** (standard 1), the **Stock Ammonia Standard** (standard 2), and the **Stock Nitrate Standard** (standard 3). Dilute to mark with **water** and mix.

6.4.2.6 **Standard 6. Working Nitrite Standard - 20 mg N/L as NO_2^-**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **20 mL Stock Nitrate Standard** (standard 4). Dilute to mark with **water** and mix.

6.4.2.7 **Standard 7. Working Quality Control Standard - 32.61 mg P/L as PO_4^{3-}, 31.06 mg N/L as NH_4, and 27.11 mg N/L as NO_3^-.**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the E M Science **1000 mg/L Phosphate Standard Solution** (326.1 mg P/L), **20 mL** of the E M Science **1000 mg/L Ammonia Standard Solution** (776.5 mg N/L), and **60 mL** of the E M Science **1000 mg/L Nitrate Standard Solution** (225.9 mg N/L). Dilute to mark with **water** and mix.

Note: 1000 mg/L standards by other reputable laboratory vendors may be substituted.

6.4.2.8 **Calibration Standards**

Standards are diluted to **500 mL** with **water.**

	Calibration Standards Concentration mg/L	Prepared From Concentration mg/L	Prepared From Aliquot mL
1	20.00	50	200
2	10.00	50	100
3	4.00	50	40
4	2.50	50	25
5	1.00	10	50
6	0.10	1	50
7	0.02	0.10	100
8	0.00	Water	0

For standards for samples that have 1 mL of 1 N H_2SO_4 added per 100 mL, add **5 mL** of **1N H_2SO_4** to each standard after building to volume.

Note: If other acid concentrations are used to preserve samples, match for standards.

6.4.2.9 **Cadmium Reduction Column Efficiency Check Standard - 2.00 mg N/L as NO_2^-**

In a **500 mL** volumetric flask add about **300 mL water**. Pipette **50 mL** of the **Working Nitrite Standard** (standard 6). Dilute to mark with **water,** add **5 mL** of **1N H_2SO_4** and mix.

6.4.2.10 **Laboratory Control Standard - 1.63 mg P/L as PO_4, 1.55 mg N/L as NH_3, and 1.36 mg N/L as NO_3^-.**

In a **1 L** volumetric flask add about **700 mL water**. Pipette **50 mL** of the **Working Quality Control Standard** (standard 7). Dilute to mark with **water,** add **10 mL** of **1N H_2SO_4** and mix.

6.5 Quality Control Sample Requirements

Begin and end each run by measuring a laboratory control standard, a midpoint calibration standard run as a sample, and a reagent blank. When the run is long enough, every twentieth sample should be followed by the above three QC check samples. Recovery should be 90 to 110% of the expected value.

7.0 PROCEDURE

7.1 Procedure Instructions

7.1.1 The instrument is calibrated each day of use and may be calibrated with each sample tray.

7.1.2 Prepare reagents and standards as described in section 6.4.

7.1.3 Set up manifold as shown in section 9.2.

7.1.4 Enter data system parameters as in section 9.1.

7.1.5 Pump deionized water through all reagent lines and check for leaks and smooth flow. Allow 15 minutes for heating unit to warm up to 37°C. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

7.1.6 Pour samples and standards into vials. If samples have particulate matter, filter them into the sample vial with a syringe and nylon syringe filter. Load standard and sample trays.

7.1.7 Place samples and standards in the autosampler. Enter the information required by the data system, such as standard concentration, and sample identification.

7.1.8 Calibrate the instrument by injecting the standards. The data system will then associate the concentration with the instrument responses for each standard.

7.1.9 If samples require color correction, inject the samples with color development, then inject the samples with water replacing the color reagent (reagent 3).

7.1.10 At end of run, remove all transmission lines from reagents and place them in water. Pump for about five minutes.

7.1.11 Place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (Reagent 5). Pump for about 5 minutes to remove any precipitated reaction products.

7.1.12 Remove the reagent lines from the NaOH - EDTA solution and place them in water. Pump for an additional 5 minutes.

7.1.13 Remove the transmission lines from the water and pump all lines dry.

7.2 Calculations and Recording Data

7.2.1 Calibration is done by injecting standards. The data system will then automatically prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation provided by the software.

7.2.2 Create a custom report. (Lachat Instruments, QuickChem 8000 Automated Ion Analyzer Omnion FIA Software Installation and Tutorial Manual, page 43, Task 11 - Creating a Custom Report)

7.2.3 Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

7.2.4 Samples that require color correction: From the value obtained with color developer added, subtract the value obtained without color developer. When a large number of samples are analyzed, use a spreadsheet to calculate the color correction.

7.2.5 Report results in mg PO_4-P/L.

8.0 SAFETY

8.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Use routine laboratory protective clothing (lab coat, gloves, and eye protection) when handling these reagents. Thoroughly wash any skin that comes into contact with any of these chemicals. Avoid creating or inhaling dust or fumes from solid chemicals.

9.0 NOTES

9.1 Data System Parameters

Method Filename:	PANHANOW.MET
Method Description:	Ortho P (a) = 4.0 to 0.02 mg P/L
	NH_3-N (a) = 20.0 to 0.1 mg N/L
	NO_2-N/NO_3-N (a) = 20.0 to 0.2 mg N/L

Analyte Data:

Analyte Name:	Orthophosphate (PO_4)-P
Concentration Units:	mg PO_4-P/L
Chemistry:	Direct
Inject to Peak Start (s):	10.0
Peak Base Width (s):	23.000
% Width Tolerance:	100.000
Threshold:	5000.000
Autodilution Trigger:	Off
QuickChem Method:	10-115-01-1-A

Calibration Data:

Levels: (mg NO_3-N/L)	3: 4.000	4: 2.500	5: 1.000
	6: 0.100	7: 0.020	8: 0.000
Calibration Rep Handling:	Average		
Calibration Fit Type:	1st Order Poly		
Force through Zero:	No		
Weighing Method:	None		
Concentration Scaling:	None		

Sampler Timing:

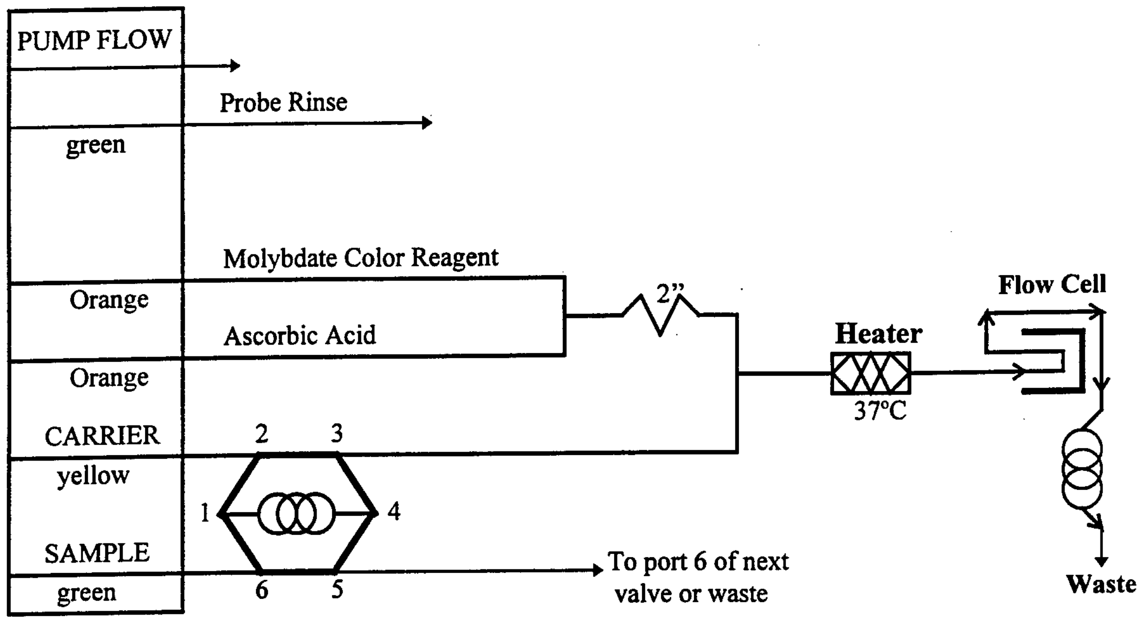
Method Cycle Period:	70.0
Min. Probe in Wash Period:	9.0
Probe in Sample Period:	30.0

Valve Timing:

Method Cycle Period:	70.0
Sample Reaches 1st Valve:	18.0
Valve:	On
Load Time:	0.0
Load period	25.0
Inject Period:	45.0

Sample Loop: 75.5 cm

9.2 Orthophosphate Manifold Diagram



Sample Loop = 75.5 cm

Interference Filter = 880 nm

Carrier is DI Water

All manifold tubing is **0.8 mm (0.32 in) i.d.** Lachat Part No. 50028. This is **5.2 uL/cm**.

2 is **135 cm** of tubing on a **7 cm** coil support.

Apparatus: The  37°C includes 175 cm of tubing wrapped around the heater block at 37°C. An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required.

10.0 ATTACHMENTS AND APPENDICES

None

End of Procedure

APPENDIX A-13

Procedure for Total Phosphorus: Method 10-115-01-1-C

WP-0022 - Total Phosphorus by Flow Injection Analyzer
June 19, 1997

1.0 Procedure

Perform analysis for total phosphorus in accordance with the procedure for Lachat Quick Chem 8000 flow injection analyzer as attached.

2.0 Recordkeeping

retain all machine printouts, analysis worksheets, preparation worksheets, percent recovery calculation of quality control samples, standards preparation log, and notes as quality assurance records.

3.0 Quality Control Samples

For each batch of samples, perform a method blank, reagent blank, and a calibration check sample. For each batch, introduce one quality control sample made from a separate stock than that used to calibrate the machine. Where possible, for each batch analyze one matrix spike sample. For each batch analyze a matrix spike duplicate or a sample duplicate.

William J. Rogers

Quality Assurance Officer

LACHAT

INSTRUMENTS

QuikChem Method 10-115-01-1-C

Total Phosphorus in Kjeldahl Digests

0.01 to 5.0 mg P/L

-- Principle --

Water samples are digested with sulfuric acid in a block digestor. Using a mercuric oxide catalyst, the samples' phosphorus is converted to the orthophosphate anion. Potassium sulfate is also added to raise the boiling temperature of the digestion and speed the conversion to orthophosphate. The digest is diluted with water.

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

-- Interferences --

1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.
2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.
3. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

-- Special Apparatus --

1. Heating Unit
2. Block Digestor/75 mL (Lachat Part No. 1800-000)

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QUIKCHEM METHOD 10-115-01-1-C

DETERMINATION OF TOTAL PHOSPHORUS BY FLOW INJECTION ANALYSIS COLORIMETRY

1. SCOPE AND APPLICATION

1.1. This method covers the determination of total phosphorus in Kjeldahl digests.

1.2. The method is based on reactions that are specific for the orthophosphate ion.

1.3. The applicable range is 0.01 to 5.0 mg P/L. The method detection limit (MDL) is 0.003 mg P/L. Approximately 60 samples per hour can be analyzed.

2. SUMMARY OF METHOD

2.1. Water samples are digested with sulfuric acid in a block digestor with a mercuric oxide catalyst, the samples' phosphorus is converted to the orthophosphate anion. Potassium sulfate is also added to raise the boiling temperature of the digestion and speed the conversion to orthophosphate. The digest is diluted with water.

2.2. The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3. DEFINITIONS

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY FORTIFIED BLANK (LFB) -- an aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY FORTIFIED SAMPLE MARIX (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9. METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.11. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.12. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) -- 26 West Martin Luther King Drive, Cincinnati, Ohio 45268, (513) 569-7453.

4. INTERFERENCES

4.1. Silica forms a pale blue complex which also absorbs at 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.

4.2. Concentrations of ferric iron greater than 50 mg/L will cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Samples high in iron can be pretreated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

4.3 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

5. SAFETY

5.1. The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.

5.3.1. Mercury

5.3.2. Sulfuric acid

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special apparatus

6.4.1. Heating Unit

6.4.2. Block Digestor/75 mL (Lachat Part No. 1800-000)

6.4.3. 5 mL and 20 mL repipet dispensers

6.4.4. Vortex mixer

7. REAGENTS AND STANDARDS

7.1 PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

Degassing with helium:

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140 kPa (20 lb/in^2) through a helium degassing tube (Lachat Part 50100). Bubble He vigorously through the solution for one minute.

Reagent 1. Stock Mercuric Sulfate Solution

To a **100 mL** volumetric flask, add **40 mL water, 10 mL concentrated sulfuric acid** (H_2SO_4), and **8 g red mercuric oxide** (HgO). Stir with a magnetic stirrer at low heat until dissolved, dilute to the mark, and invert to mix. Store up to two months.

Reagent 2. Digestion solution

In a **1 L** volumetric flask, add approximately **700 mL water**, then add **200 mL concentrated sulfuric acid** (H_2SO_4). Add **133 g potassium sulfate** (K_2SO_4). Add **25 mL Stock Mercuric Sulfate Solution (Reagent 1)** and dilute to the mark. Mix with a magnetic stirrer and allow the solution to cool. Dilute to the mark after the solution has cooled. Prepare fresh monthly.

Reagent 3. Diluent 4.8% Sulfuric acid (For simulated standards)

By Volume: In a **1 L** volumetric flask containing approximately **600 mL water,** add **240 mL Reagent 2** (Digestion Solution). Dilute to the mark and invert to mix.

By Weight: To a tared **1 L** container, add **760 g water** and **240 mL Reagent 2** (Digestion Solution). Invert to mix.

Reagent 4. Stock Ammonium Molybdate Solution

By Volume: In a **1 L** volumetric flask dissolve **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in approximately **800 mL water.** Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store up to two months in plastic and refrigerate.

By Weight: To a tared 1 L container add **40.0 g ammonium molybdate tetrahydrate** [$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] and 983 g water. Mix with a magnetic stirrer for at least four hours. Store up to two months in plastic and refrigerate.

Reagent 5. Stock Antimony Potassium Tartrate Solution

By Volume: In a **1 L** volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6 \cdot 1/2H_2O$) in approximately **800 mL water.** Dilute to the mark and mix with a magnetic stirrer until dissolved. Store, up to two months, in a dark bottle and refrigerate.

By Weight: To a **1 L** dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6 \cdot 1/2H_2O$) and **995 g water.** Mix with a magnetic stirrer until dissolved. Store, up to two months, in a dark bottle and refrigerate.

Reagent 6. Molybdate Color Reagent

By Volume: To a **1 L** volumetric flask add about **500 mL water,** and then add **213 mL Ammonium Molybdate Solution (Reagent 4)** and **72 ml Antimony Potassium Tartrate Solution (Reagent 5).** Dilute to the mark and invert to mix. Degas with helium. Prepare weekly.

By Weight: To a tared **1 L** container add **715 g water,** and then **213 g Ammonium Molybdate Solution (Reagent 4)** and **72.0 g Antimony Potassium Tartrate Solution (Reagent 5).** Shake and degas with helium. Prepare weekly.

Reagent 7. Ascorbic Acid Reducing Solution Always Degas

By Volume: In a **1 L** volumetric flask dissolve **60.0 g ascorbic acid** in about **700 mL water.** Dilute to the mark and mix with a magnetic stirrer. Degas this solution with helium. Add **1.0 gm SDS** (sodium dodecyl sulfate Aldrich catalog no. 86,201-0). Mix with a magnetic stirrer. Prepare fresh every two days. Degas before add last

By Weight: To a tared **1 L** container, add **60.0 g ascorbic acid** and **975 g water.** Mix with a magnetic stirrer until dissolved. Degas this solution with helium. Add **1.0 gm SDS** (sodium dodecyl sulfate, Aldrich catalog no. 86,201-0). Mix with a magnetic stirrer. Prepare fresh every two days.

Reagent 8. Sodium Chloride/Sodium Hydroxide Solution

By Volume: In a **1 L** volumetric flask dissolve **160 g sodium chloride** and **20 g sodium hydroxide** in about **600 ml water.** Dilute to the mark and mix with a magnetic stirrer. Degas this solution with helium. Prepare weekly.

By Weight: To a tared **1 L** container, add **160 g sodium chloride** and **20 g sodium hydroxide,** and **916 g water.** Mix with a magnetic stirrer until dissolved. Degas this solution with helium. Prepare weekly.

Reagent 9. Sulfuric Acid/Potassium Sulfate solution (Carrier)

The sulfuric acid concentration in the carrier needs to match the digestion matrix. The table below shows the quantity of sulfuric acid required to prepare 1 L of the carrier so that it will match some of the common digestion matrices. This table assumes 5 mL of digestion solution is added to each sample. Prepare weekly.

Required Reagents

final volume of digestate (mL)	*sulfuric acid (%v/v)*	potassium sulfate (K_2SO_4)(g)	DI water (mL)	**By Weight** sulfuric acid (H_2SO_4) g	**By Volume** sulfuric acid (H_2SO_4) (mL)
20	*5.0*	31.7	938	92.0	50
21*	***4.8***	**31.7**	**940**	**88.3**	**48**
25	*4.0*	31.7	948	73.6	40

* used in Lachat Method Support Data

Reagent 10. Sodium Hydroxide - EDTA Rinse

Dissolve **65 g sodium hydroxide** (NaOH) and **6 g tetrasodium ethylenediamine tetraacetic acid** (Na4EDTA) in 1.0 L or 1.0 kg water. Prepare fresh monthly.

7.2 PREPARATION OF STANDARDS

Non-Digested Standards

Standard 1. Stock Standard 250.0 mg P/L

In a 1 L volumetric flask dissolve **1.099 g primary standard grade anhydrous potassium dihydrogen phosphate** (KH_2PO_4) that has been dried for two hours at 110°C in about **800 mL water.** Dilute to the mark with **DI water** and invert to mix.

Standard 2. Working Stock Standard Solution 5.00 mg P/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **Diluent** (Reagent 3). Invert to mix.

Note:

Non-Digested standards will need to be labeled to reflect the changing concentration or dilution which occurs during the digestion procedure. The following formula can be used to calculate the adjustment. For example, using a final volume of 21 mL for the digestate and an initial sample volume of 20 mL results in a labeled concentration of a 5.25 mg P/L for a 5.00 mg P/L non-digested standard. *If the digestion volumes used by your laboratory require the digested equivalent adjustment, the digested equivalents will be the values entered into the data system (e.g. 5.25 rather than 5.00 mg P/L).*

$$\text{Labeled non-digested standard concentration} = \frac{\text{final digestate volume}}{\text{initial sample volume}} \times \text{standard concentration}$$

These standards will not be digested.

Working Standards (Prepare Daily)	A	B	C	D	E	F
Concentration (mg P/L)	5.00	2.00	1.00	0.50	0.10	0.00

By Volume

Volume (mL) of Standard 2 diluted to 250 mL with Reagent 3	250	100	50	25	5	0

By Weight

Weight (g) of Standard 2 diluted to final weight (~250 g) divide by factor below with Reagent 3.	250.0	100	50	25	5	0
Division Factor Divide exact weight of the standard by this factor to give final weight	1.00	0.40	0.20	0.10	0.02	0

Digested Standards

Standards prepared in DI water must be carried through the digestion procedure (see section 11).

Standard 3. Working Stock Standard Solution 5.00 mg P/L

By Volume: In a **250 mL** volumetric flask, dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **DI water**. Invert to mix.

By Weight: To a tared **250 mL** container add about **5.0 g Stock Standard** (Standard 1). Divide the actual weight of the solution added by **0.02** and make up to this resulting total weight with **DI water**. Invert to mix.

These standards **will be** digested

Working Standards (Prepare Daily) Concentration mg P/L	**A** 5.00	**B** 2.00	**C** 1.00	**D** 0.50	**E** 0.10	**F** 0.00

By Volume

Volume (mL) of **Standard 3** diluted to 250 mL with DI water.	250	100	50	25	5	0

By Weight

Weight (g) of **Standard 3** diluted to final weight (~250 g) divide by **factor** below with DI water.	250.0	100	50	25	5	0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.00	0.40	0.20	0.10	0.02	0

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2. Samples may be preserved by addition of a maximum of 2 mL of concentrated H_2SO_4 per liter to produce a pH less than 2 and stored at 4^oC. Acid preserved samples have a holding time of 28 days. Sample digests should be run within one week of digestion.

9. QUALITY CONTROL (USEPA GUIDELINE)

9.1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every six months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within +/-10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = tS$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty one replicates]. S = standard deviation of the replicate analyses.

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3. The laboratory must used LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \overline{X} + 3S$$

$$\text{LOWER CONTROL LIMIT} = \overline{X} - 3S$$

The optional control limits must be equal to or better than the required control limits of 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going

precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of analyte to a minimum of 10% of routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.2. Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \text{ X100}$$

Where, R = percent recovery

C_s = fortified sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.4. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in Section 7.2).

10.2. Calibrate the instrument as described in Section 11.

10.3. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.4. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed +/-10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. DIGESTION PROCEDURE

11.1.1. Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.2. To a **20.0 mL** sample add **5 mL digestion solution** (Reagent 2) and mix. This is efficiently accomplished using an acid resistant 5 mL repipet device (EM Science, 108033-1, available through major scientific supply companies.)

11.1.3. Add two to four Hengar granules to each tube. Hengar (Alundum) granules are effective for smooth boiling. They are available from Fisher Scientific, cat. no. S145-500. Teflon boiling chips may be used as an alternative.

11.1.4. Place tubes in the preheated block digestor for one hour at 160°C. Water from the sample should have boiled off before increasing the temperature in step 11.1.6.

11.1.5. Place the cold fingers on the top of the sample tube.

11.1.6. Continue to digest for 1.5 additional hours with the controller set to 380 ^{O}C. This time includes the ramp time for the block temperature to come up to 380

^{o}C. The typical ramp time is 50-60 minutes and 380 ^{o}C must be maintained for 30 minutes.

11.1.7. Remove the samples from the block and allow about 10 minutes to cool.

11.1.8. Add **20.0 mL** water to each tube and vortex to mix. The final volume should be **21 mL.**

11.1.9. Transfer the digestate into a clean labeled container. If samples are not run immediately they should be covered tightly.

11.2. SYSTEM START-UP PROCEDURE

11.2.1. Prepare reagent and standards as described in Section 7.

11.2.2. Set up manifold as shown in Section 17.1.

11.2.3. Input peak timing and integration window parameters as suggested in Section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place standards in the sampler, and fill the sample tray. Input the information required by data system, such as concentration, replicates and QC scheme.

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with responses for each standard.

11.2.7. After a stable baseline has been obtained, start the sampler and perform analysis.

11.3. SYSTEM NOTES

11.3.1. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

11.3.2. Allow 15 min for heating unit to warm up to 37°C.

11.3.3. If necessary, at end of run place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (Reagent 10). Pump this solution for approximately five minutes to remove any precipitated reaction products. Then place these lines in water and pump for an additional five minutes. Then pump dry all lines.

11.3.4. If sample concentrations are greater than the high standard, the digested sample should be diluted with **diluent** (Reagent 3). When the digital diluter is used, **Diluent** (Reagent 3) should be used. Do not dilute digested samples or standards with DI water.

11.3.5. To analyzing digestion sample, prepare a standard curve by plotting heights of digested standards against concentration values. Compute the concentrations by comparing the sample peak heights with the standard curve.

11.3.6. If simulation standards are used to prepare a standard curve, the response curve has to be obtained by comparing to a digested standard curve.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

12.3. Report results in mg P/L.

13. METHOD PERFORMANCE

13.1. The method performance data are presented as method support data in Section 17.2. This data was generated according to Lachat Standard Operating Procedure J001WI, Lachat FIA Support Data Generation.

14. POLLUTION PREVENTION

14.1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Society's

Department of Government Regulations and Science Policy", 115 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. The USEPA requires that laboratory waste management practice be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods, and bench operations, complying with the letter and spirit of any waster discharge permit and regulations, and by complying with all solid and hazardous waste regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in Sect. 14.3.

16. REFERENCES

16.1. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 365.4

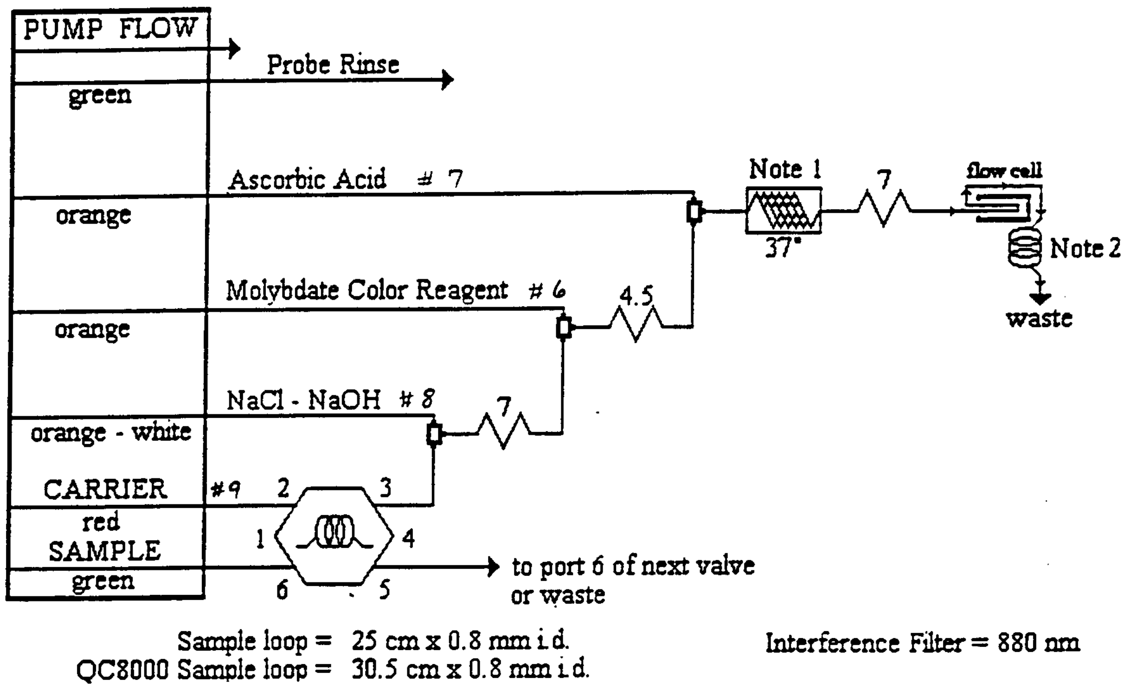
16.2. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

16.3. Lachat Instruments, QuikChem Method 10-115-01-1-C revised by Ann Zuehlke and Kevin Switala on 13 July 1992.

16.4. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. TOTAL KJELDAHL PHOSPHORUS MANIFOLD DIAGRAM:



CARRIER is sulfuric acid/potassium sulfate solution (Reagent 9).

4.5 is **70.0** cm of tubing on a 4.5 cm coil support

7 is **135** cm of tubing on a 7 cm coil support

APPARATUS: Standard valve, flow cell, and detector head modules are used.

All manifold tubing is **0.8 mm (0.032 in.) i.d.** This is **5.2 uL/cm.**

Note 1: 175 cm of heated tubing.

Note 2: 2 meter restrictor coil, 0.52 mm (0.022 in.) i.d.

17.2 Data System Parameters for the QuikChem AE

Sample throughput:	60 samples/hour; 60 s/sample
Pump speed:	35
Cycle Period:	60 s
Inject to start of peak period:	28 s
Inject to end of peak period:	86 s
Presentation, Data Window	
Top Scale Response:	0.50 abs
Bottom Scale Response:	0.00 abs
Segment/Boundaries:	A: 5.00 mg P/L
	E: 0.10 mg P/L
	F: 0.00 mg P/L

Series 4000/System IV Settings: Gain = 330 x 1

17.3. QUIKCHEM AE SUPPORT DATA

17.3.1. Support Data for Non-Digested Standards

QuikChem AE Calibration Report for Calibration 93081002
Method: TKP low level
This calibration was first done or last modified on 08/10/93 at 02:38 pm
This report prepared on 08/10/93 at 05:14 pm

	Analyte	Units	Average Concentrations: Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A,	TKP	mg P/L	5.000	4.993	0.14	0.3844
Standard B,	TKP	mg P/L	2.000	2.014	-0.71	0.1251
Standard C,	TKP	mg P/L	1.000	1.009	-0.93	0.0777
Standard D,	TKP	mg P/L	0.500	0.498	0.48	0.0383
Standard E,	TKP	mg P/L	0.100	0.100	0.00	0.0067
Standard F,	TKP	mg P/L	0.000	0.000	***.**	-0.0002

This calibration may have been modified since it was first run
If there is any question about this, see the calibration graph displayed
in "Calib Graphs and Stats" menu selection, Results/Approval.

——— End of Calibration Report ———

Calibration Statistics Report 08/10/93 05:19 pm

Cal Ref: 93081002
Method: TKP

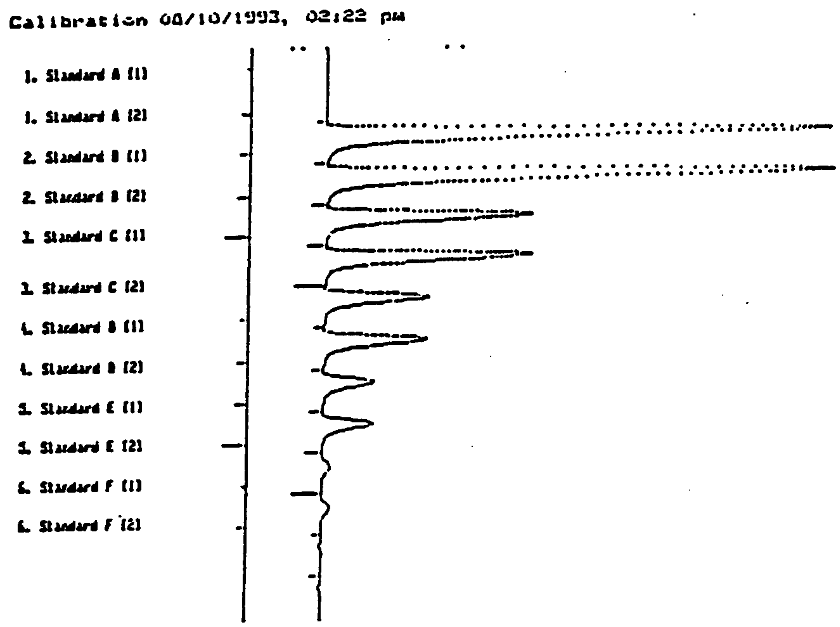
Channel: TKP

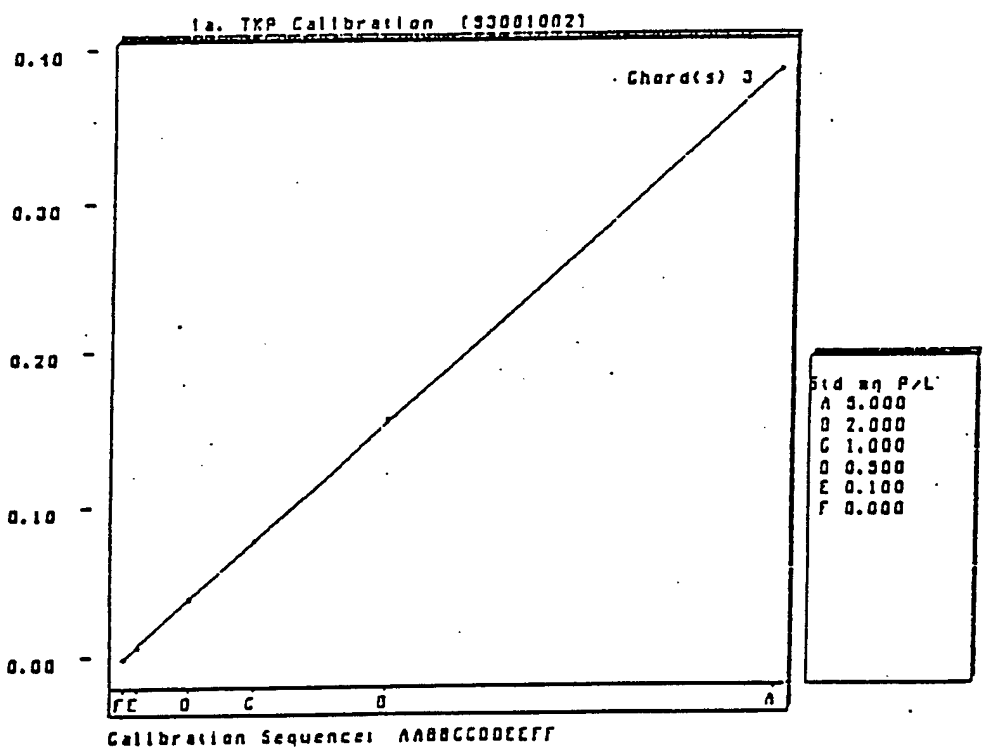
Correlation Coefficients

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.9999	0.9555 ?	0.9999	1.0000	0.9994	0.9200 ?
2	E-F	0.9986	0.1017 ?	0.9979	0.9999	0.9927	0.6885 ?

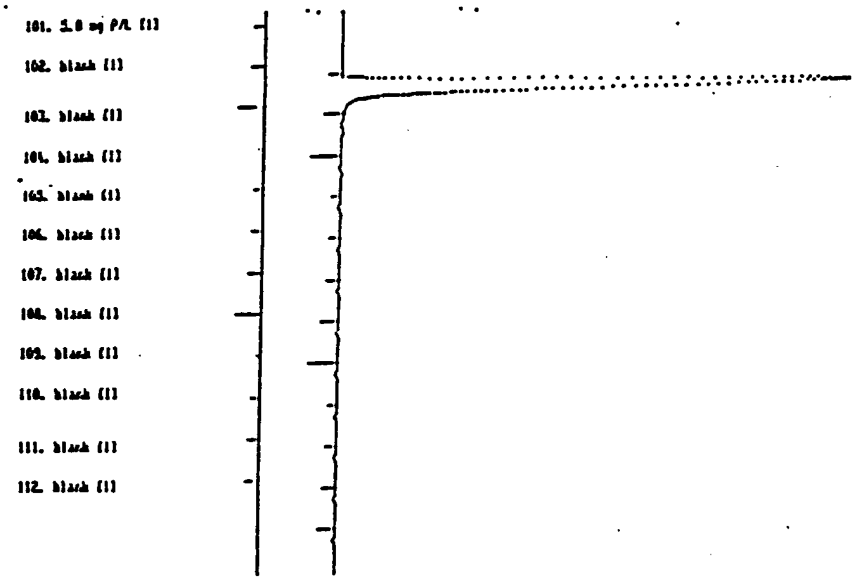
Percent Standard Deviation in Slope

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.3	7.6	0.3	0.2	0.9	10.4
2	E-F	2.6	210.2 ?	3.3	0.7	6.1	47.6


Calibration 08/10/1993, 02:22 pm
1. Standard A (1)
1. Standard A (2)
2. Standard B (1)
2. Standard B (2)
3. Standard C (1)
3. Standard C (2)
4. Standard D (1)
4. Standard D (2)
5. Standard E (1)
5. Standard E (2)
6. Standard F (1)
6. Standard F (2)


1a. TKP Calibration (930810082)
Chord(s) 3
0.40
0.30
0.20
0.10
0.00
FE D C B A
Calibration Sequence: AABBCCDDEEFF
Std mg P/L
A 5.000
B 2.000
C 1.000
D 0.500
E 0.100
F 0.000

Rack 1 (Ref: 93081002) 08/10/1993, 02:33 pm



TKP(simulated)

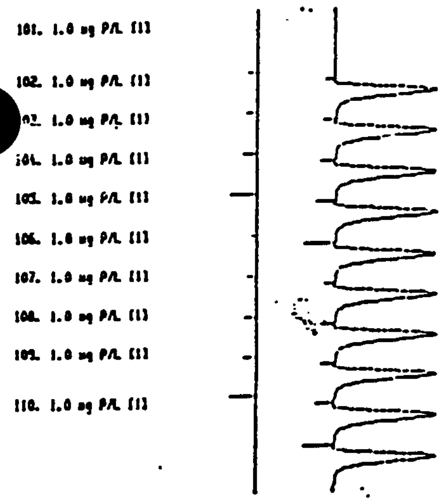
mg P/L

4.994
-0.014
0.000
-0.001
-0.001
0.001
0.001
0.001
-0.001
0.001
0.001
0.000

Carryover:
x = 0.000 mg P/L
s = 0.001 mg P/L
t = 2.262
95%CI = 0.000 +/- 0.001 mg P/L

EMDL:
s = 0.001 mg P/L
t = 4.65
EMDL = 0.005 mg P/L

Rack 1 (Ref: 93081005) 08/10/1993, 03:35 pm

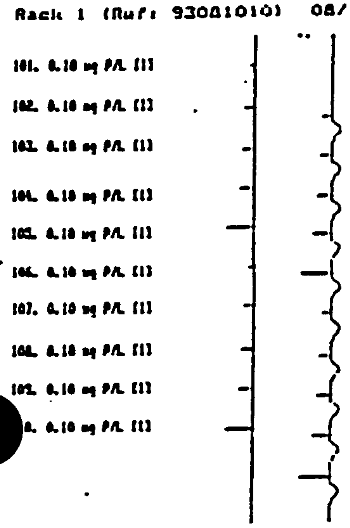


mg P/L

1.006
1.003
1.010
1.011
1.009
1.009
1.013
1.012
1.011
1.011

Precision:
x = 1.010 mg P/L
s = 0.003 mg P/L
%RSD = 0.3%

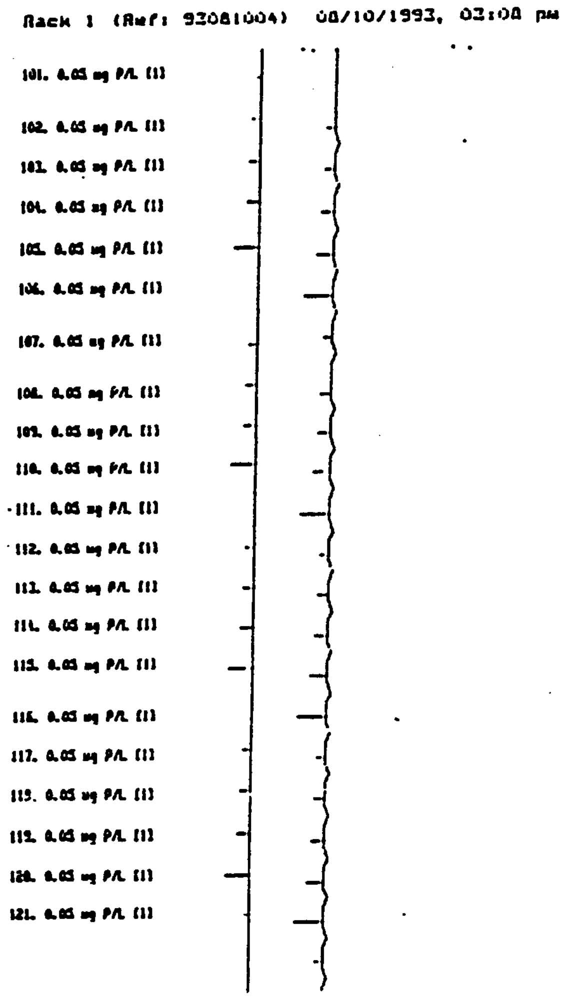
Rack 1 (Ref: 93081010) 08/10/1993, 04:59 pm



mg P/L

0.103
0.100
0.103
0.102
0.102
0.100
0.101
0.101
0.102
0.102

MDL:
x = 0.102 mg P/L
s = 0.001 mg P/L
t = 2.821
MDL = 0.003 mg P/L



mg P/L
0.049
0.048
0.049
0.048
0.049
0.048
0.051
0.049
0.049
0.048
0.048
0.048
0.049
0.048
0.047
0.046
0.049
0.048
0.049
0.048
0.048

MDL:

$x = 0.048$ mg P/L

$s = 0.001$ mg P/L

$t = 2.528$

MDL = 0.003 mg P/L

17.3.2. Support Data for Digested Standards

QuikChem AE Calibration Report for Calibration 93081003
Method: TKP low level
This calibration was first done or last modified on 08/10/93 at 04:04 pm
This report prepared on 08/10/93 at 05:25 pm

	Analyte	Units	Average Concentrations — Known	Determined	% Residual	Baseline Corrected Average Absorbance
Standard A,	TKP	mg P/L	5.000	4.992	0.15	0.3425
Standard B,	TKP	mg P/L	2.000	2.019	-0.93	0.1386
Standard C,	TKP	mg P/L	1.000	1.006	-0.57	0.0691
Standard D,	TKP	mg P/L	0.500	0.491	1.89	0.0338
Standard E,	TKP	mg P/L	0.100	0.100	0.00	0.0065
Standard F,	TKP	mg P/L	0.000	-0.000	***.**	0.0014

This calibration may have been modified since it was first run!
If there is any question about this, see the calibration graph displayed
in "Calib Graphs and Stats" menu selection, Results/Approval.

——— End of Calibration Report ———

Calibration Statistics Report

Cal Ref: 93081003 08/10/93 05:30 pm
Method: TKP.

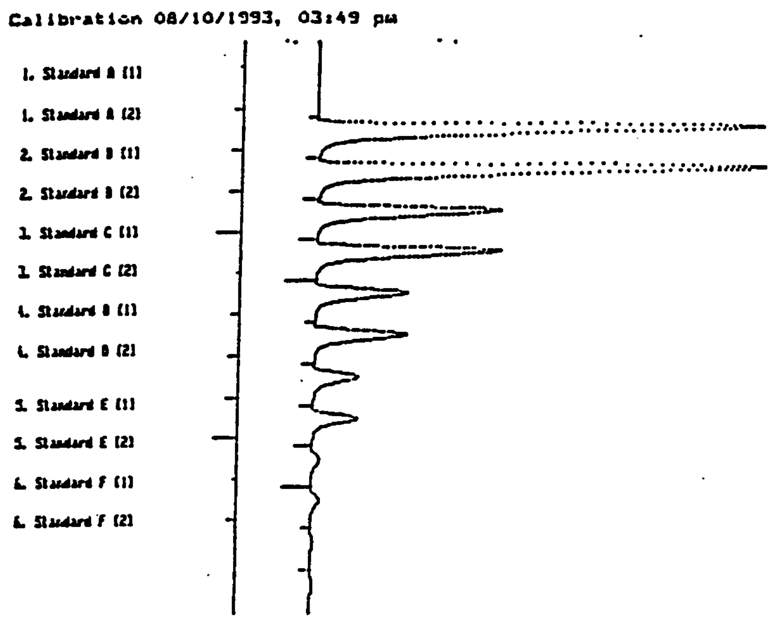
Channel: TKP

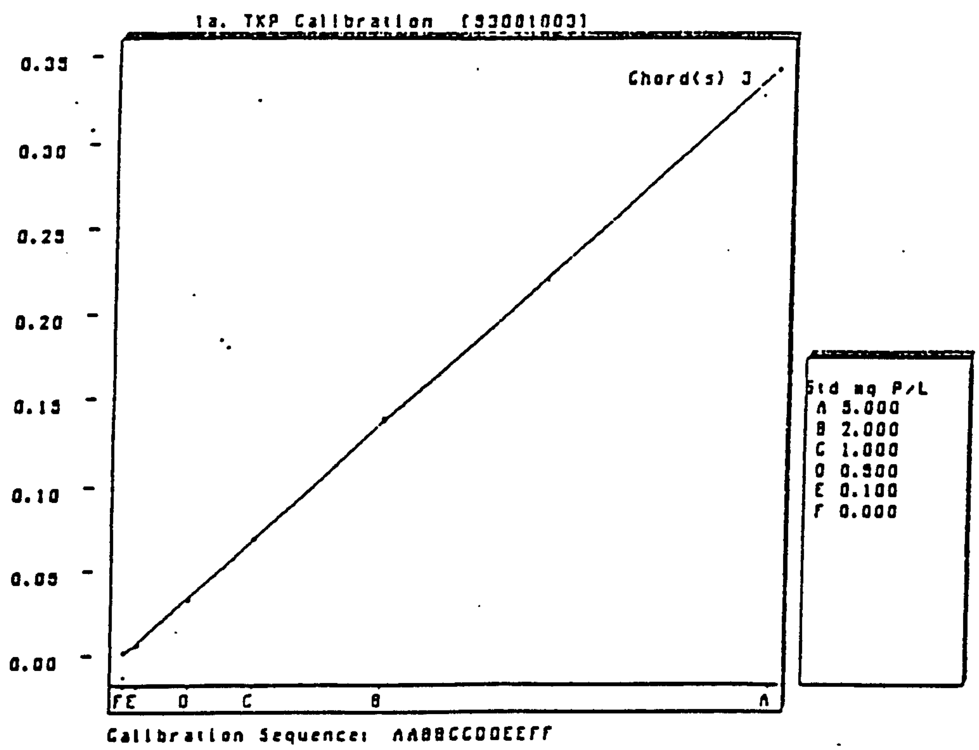
Correlation Coefficients

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.9999	0.8393 ?	0.9990	1.0000	0.9996	0.9014 ?
2	E-F	0.9977	0.3948 ?	0.9982	0.9999	0.9891 ?	0.0312 ?

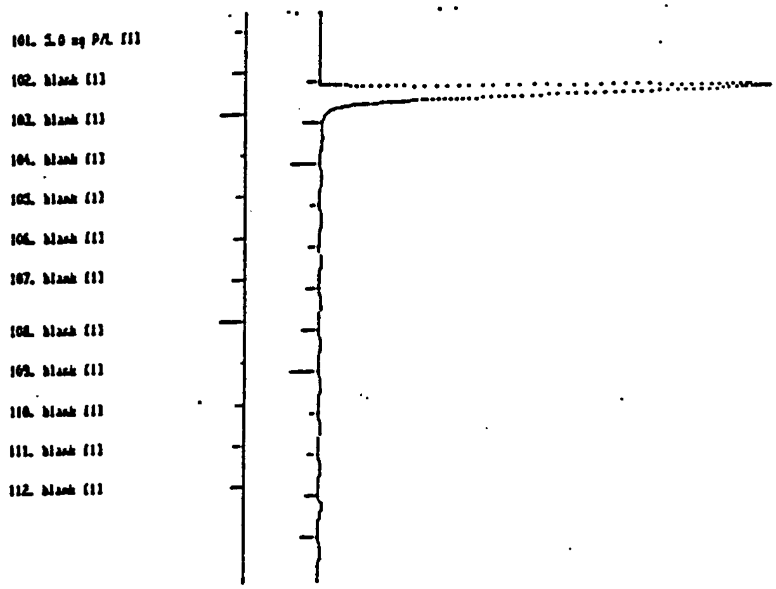
Percent Standard Deviation in Slope

Seq	Stds	Full	Chord 1	Chord 2	Chord 3	Chord 4	Chord 5
1	A-E	0.3	11.8	0.5	0.2	0.7	11.7
2	E-F	3.4	87.5 ?	3.0	0.7	7.4	394.0 ?


Calibration 08/10/1993, 03:49 pm
1. Standard A (1)
1. Standard A (2)
2. Standard B (1)
2. Standard B (2)
3. Standard C (1)
3. Standard C (2)
4. Standard D (1)
4. Standard D (2)
5. Standard E (1)
5. Standard E (2)
6. Standard F (1)
6. Standard F (2)


1a. TKP Calibration [93081003]
Chord(s) 3
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
FE D C B A
Calibration Sequence: AABBCCDDEEFF
Std mg P/L
A 5.000
B 2.000
C 1.000
D 0.500
E 0.100
F 0.000

Rack 1 (Ref: 93081006) 08/10/1993, 04:04 pm

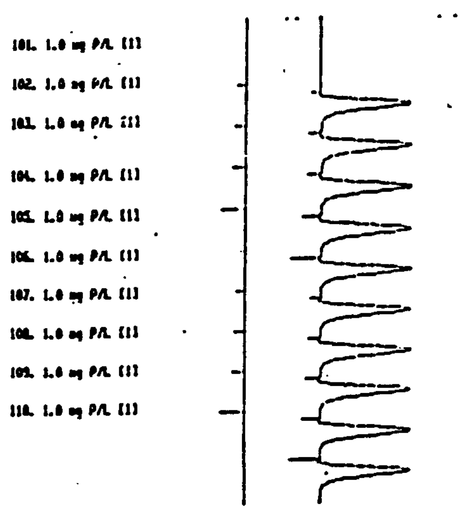


TKP(digested)
mg P/L
4.986
-0.020
-0.001
-0.001
-0.001
0.000
-0.002
-0.002
-0.002
-0.004
0.005
0.001

Carryover:
x = -0.001 mg P/L
s = 0.002 mg P/L
t = 2.262
95%CI = -0.001 +/- 0.001 mg P/L

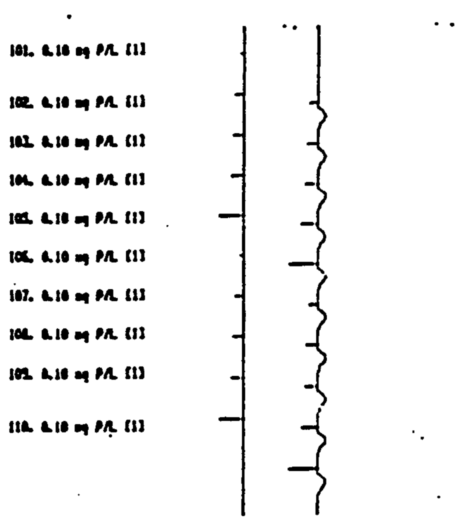
EMDL:
s = 0.002 mg P/L
t = 4.65
EMDL = 0.009 mg P/L

Rack 1 (Ref: 93081007) 08/10/1993, 04:19 pm



mg P/L
1.002
1.016
1.017
1.011
1.008
1.006
1.008
1.009
1.010
1.010

Precision:
x = 1.010 mg P/L
s = 0.004 mg P/L
%RSD = 0.4%

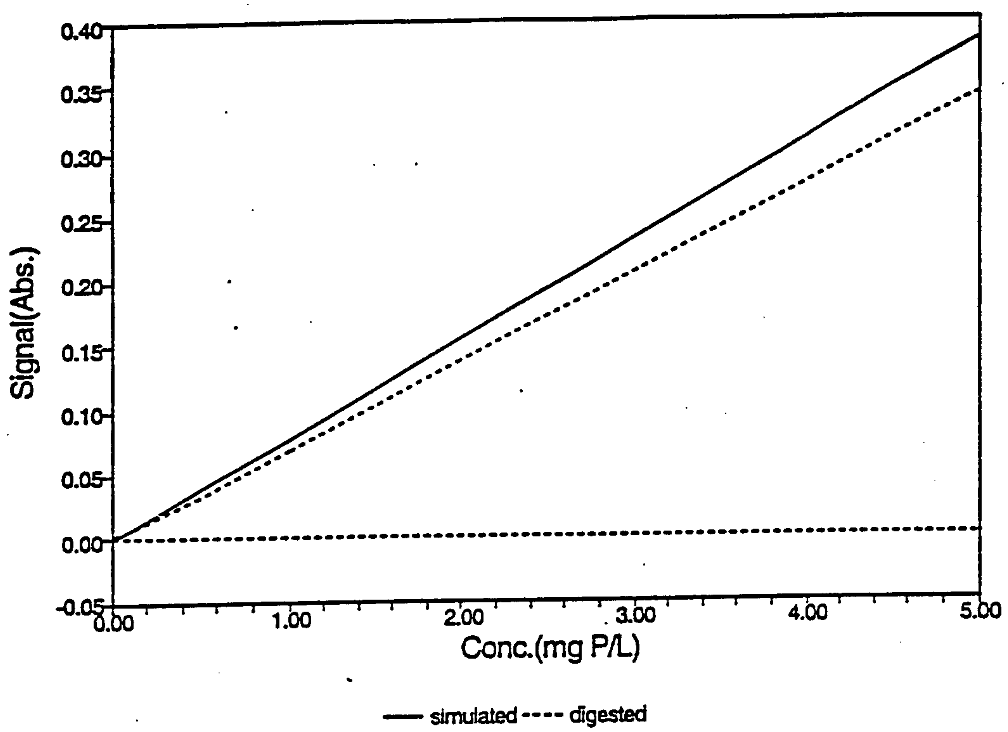


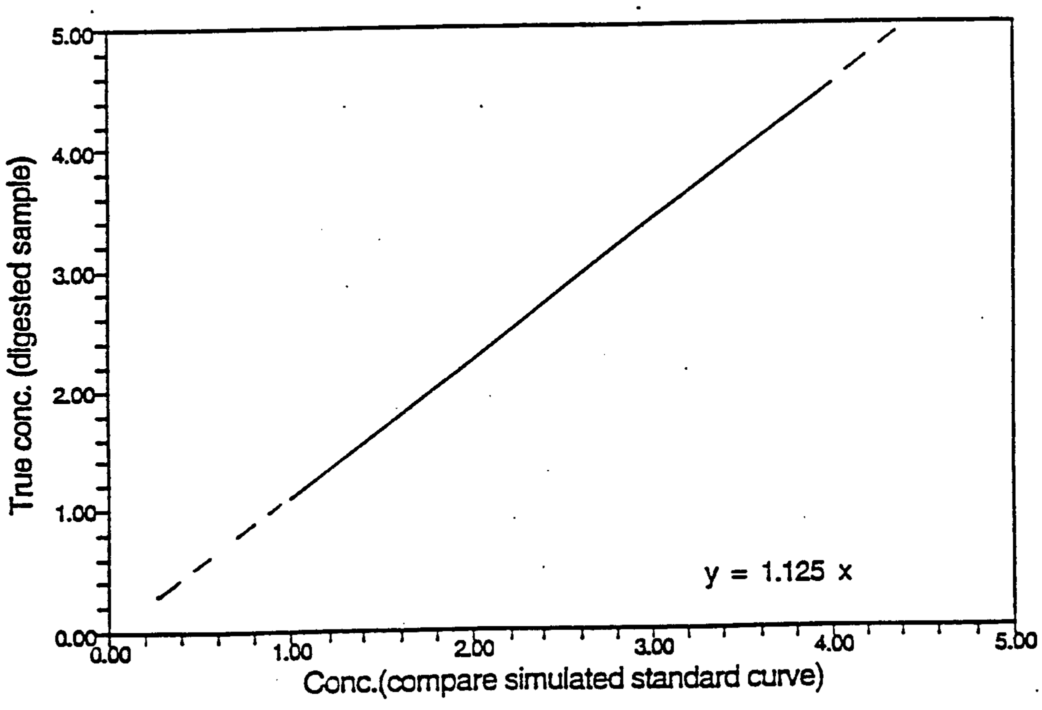
mg P/L
0.100
0.098
0.099
0.099
0.099
0.099
0.100
0.101
0.100
0.100

MDL:
x = 0.100 mg P/L
s = 0.001 mg P/L
t = 2.821
MDL = 0.003 mg P/L

Precision:
x = 0.100 mg P/L
s = 0.001 mg P/L
%RSD = 1.0%

17.3.3. Response Curve





17.4. DATA PARAMETERS FOR THE QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Analyte data:

Peak Base Width: 48 s

% Width Tolerance: 100

Threshold: 14000

Inject to Peak Start: 23.4 s

Chemistry: Direct

Calibration Data:

Levels	1	2	3	4	5	6
Concentrations mg P/L	5.00	2.00	1.00	0.50	0.10	0.00

If digested equivalents are used (see page 9 for details) enter these values in the above concentrations table.

Calibration Fit Type: 1st Order Polynomial

Weighting Method: none

Sampler Timing:

Min. Probe in Wash Period: ~~20 s~~

Probe in Sample Period: ~~20 s~~

Valve Timing:

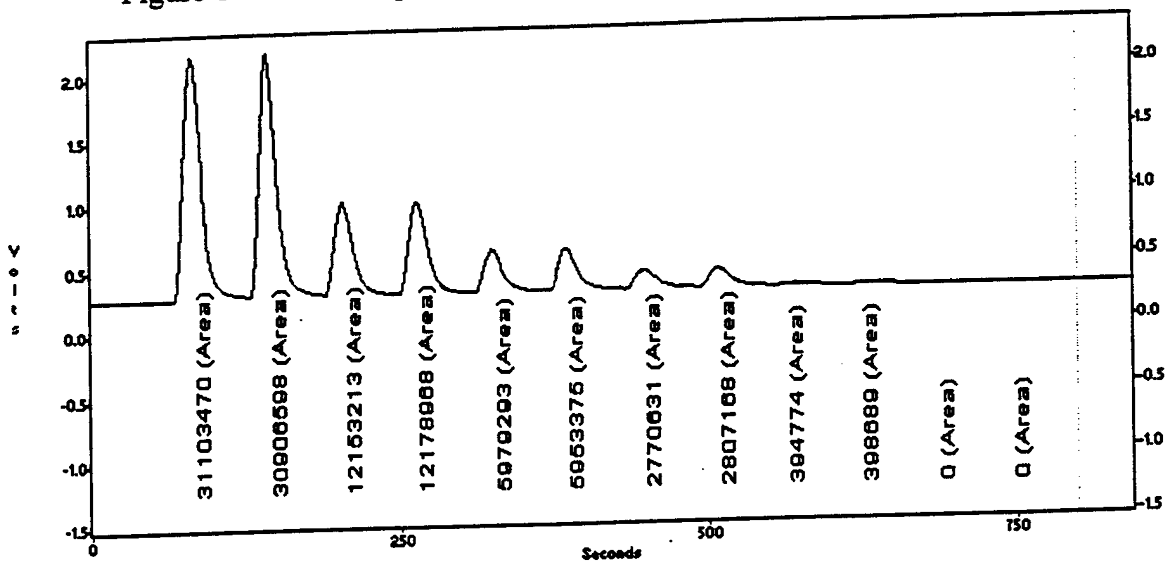
Load Period: ~~12~~ s

Inject Period: 25 s

Load Time: 0.0 s

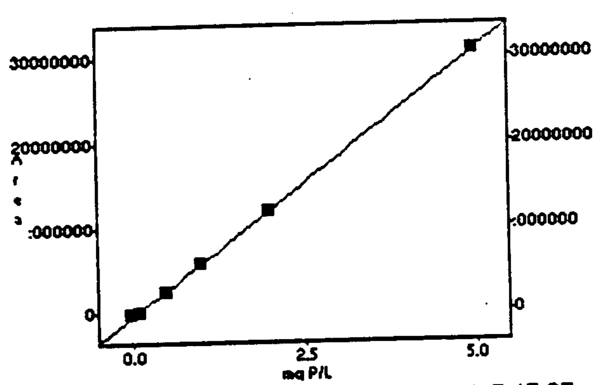
17.5. QUIKCHEM 8000 METHOD SUPPORT DATA

Figure 1. Total Phosphorus Calibration Peaks



Calibration Graph and Statistics

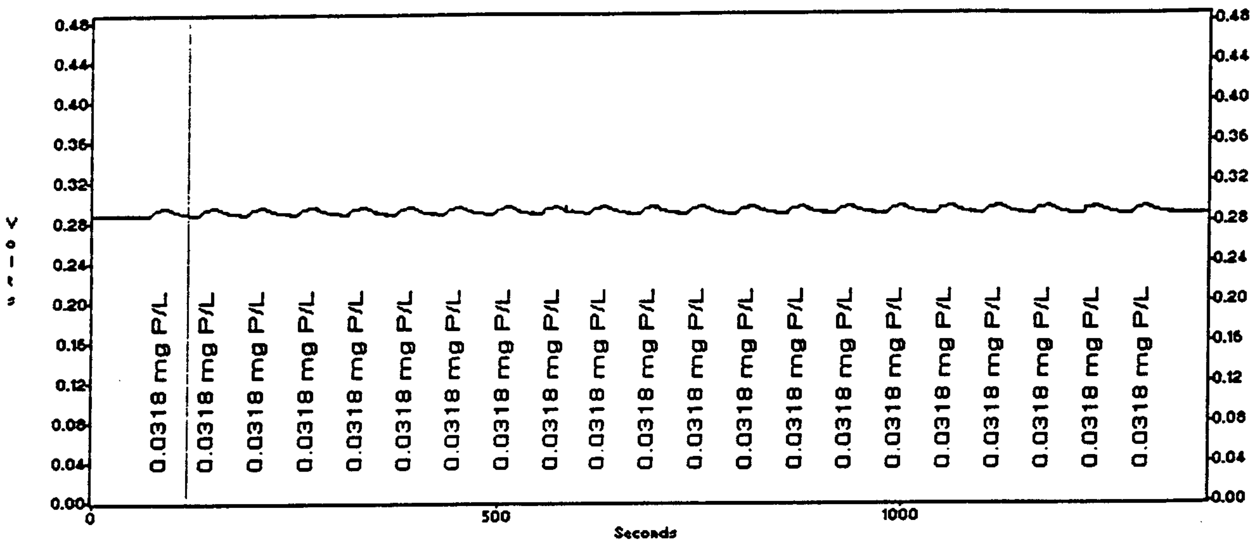
Level	Area	mg P/L	Determined	Rep 1	Rep 2	Replic STD	Replic RSD	% residual
1	30906598	5	5.001	30906598	31103470	107831.2	0.3	-0.1
2	12178968	2	1.996	12178968	12153213	14106.6	0.1	0.4
3	5953375	1	0.989	5953375	5979293	14195.9	0.2	1.1
4	2827391	0.5	0.4855	2827391	2810339	9339.6	0.3	2.9
5	412386	0.1	0.0968	412386	413693	716.1	0.2	3.2
6	0	0	0	0	0.0	0.0	0.0	—



Scaling: None
Weighting: None
1st order poly
Conc = 1.610e-007area + 3.037e-002

ACQ. TIME: Sep 8, 1994 7:47:37
DATA FILENAME: C:\OMNION\DATA\1011511C\090894C1.FDT
METHOD FILENAME: C:\OMNION\METHODS\1011511C.MET

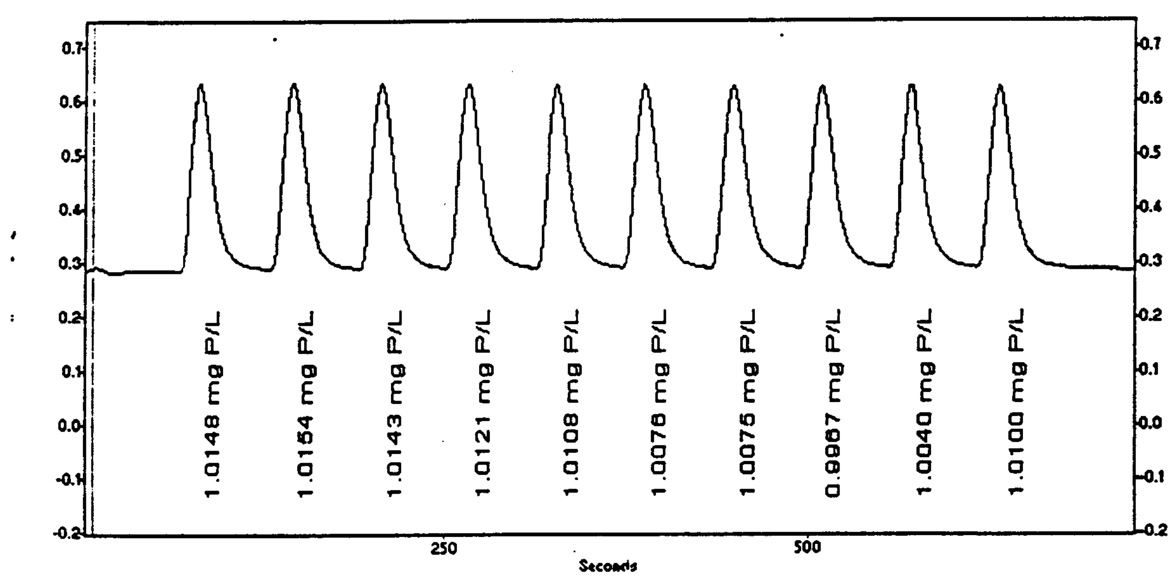
Figure 2. Method Detection Limit for Total Phosphorus



MDL = 0.0026 ug P/L
n = 20

ACQ. TIME:	Sep 8, 1994 8:05:19
DATA FILENAME:	C:\OMNION\DATA\1011511C\090894M1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1011511C.MET

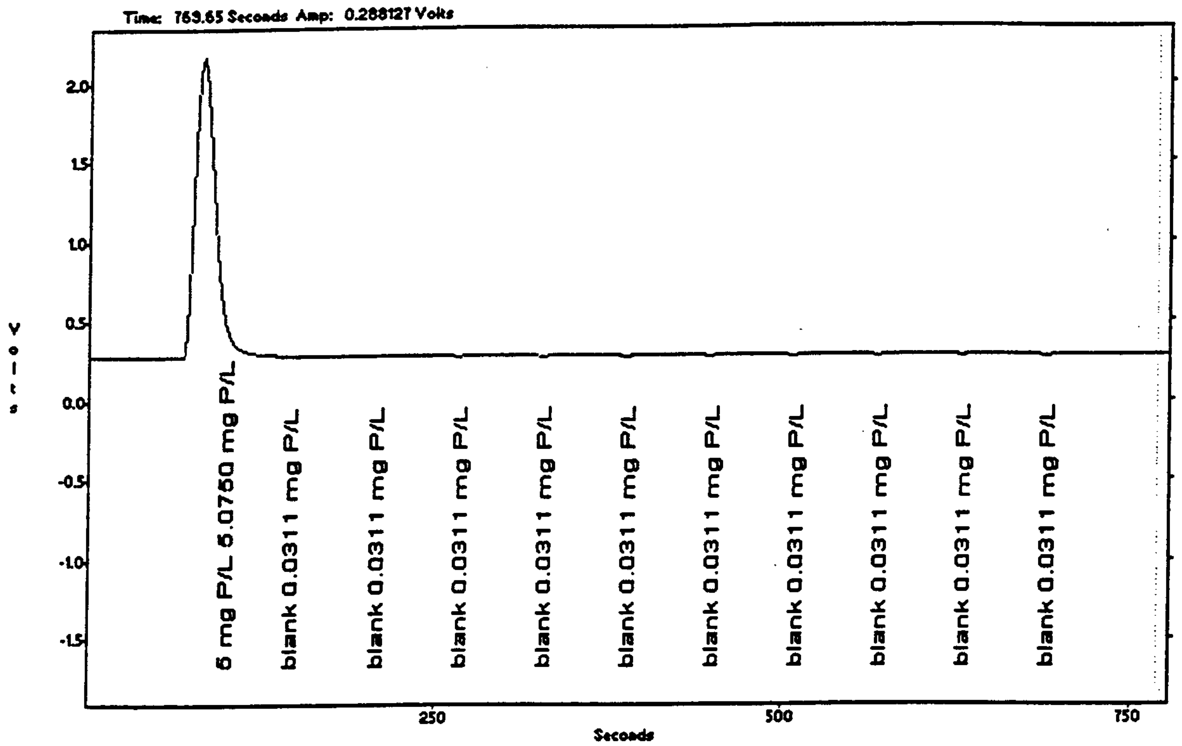
Figure 3. Precision for Total Phosphorus



Precison = 0.59 % RSD

ACQ. TIME:	Sep 8, 1994 10:02:34
DATA FILENAME:	C:\OMNION\DATA\1011511C\090894P1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1011511C.MET

Figure 4. Carryover for Total Phosphorus



Carryover passed

ACQ. TIME:	Sep 8, 1994 10:15:41
DATA FILENAME:	C:\OMNION\DATA\1011511C\090894R1.FDT
METHOD FILENAME:	C:\OMNION\METHODS\1011511C.MET

APPENDIX A-14

Lab Procedure for Chain of Custody

Tennessee Valley Authority

Analytical Laboratory of Environmental Applications
Environmental Research Center
Muscle Shoals, AL 35662

Procedure Number : SP-0001

Title: Sample Chain of Custody

	Signature	Title	Date
Prepared by:	William J. Rogers	QA Officer	11/26/96
Concurred:	Eugene A. Zarate	Laboratory Section Leader	11/26/96
Concurred:			
Concurred:			
Approved:	Joseph J. Hoagland	Manager	11/27/96

Revision	R0	R1	R2			
Control Date	29-Sep-89	10-Jan-96	29-Nov-96			

Copy No: 2 has been issued to holder on 12/3/96

"Sample Chain of Custody"

1.0 PURPOSE

This procedure provides instructions for sample custody from collection to final disposition.

2.0 SCOPE

This procedure applies to all samples collected under a sampling plan which requires documentation of sample custody.

3.0 SUMMARY

Requirements for documentation of sample collection and sample custody are specified.

4.0 REFERENCES

4.1 U. S. Environmental Protection Agency, "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," SW-846, 3rd Edition, Most Recent Update (September 1994)

4.2 "Preparation Aids for the Development of Category II Quality Assurance Project Plans," EPA/600/8-91/004, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Developent, U.S. Environmental Protection Agency, Cincinnati, OH 45268

4.3 "Preparation Aids for the Development of Category III Quality Assurance Project Plans," EPA/600/8-91/005, February 1991, Guy F. Simes, Risk Reduction Engineering Laboratory, Office of Research and Development, U.S. Enviromental Protection Agency, Cincinnati, OH 45268

4.4 "Sample Receipt, Log-in, and Data Handling", GLP-0016, Tennessee Valley Authority, Analytical Laboratory of Environmental Applications, Muscle Shoals, AL.

5.0 RESPONSIBILITIES

5.1 The laboratory team leader shall ensure that this procedure is followed.

5.2 The sampler shall follow this procedure to ensure sample integrity in the field.

5.3 The person transporting the samples shall follow the procedure to ensure sample integrity in transit.

5.4 The person receiving the samples shall follow this procedure to ensure sample integrity upon receipt and immediately following.

5.5 Laboratory analysts shall follow this procedure during sample analysis.

6.0 REQUIREMENTS

6.1 Prerequisites

6.1.1 Sample containers shall be cleaned to specifications of the sampling plan, or in their absence, to good commercial practice.

6.1.2 Sample containers shall have preservative added before sampling as required by the sampling plan.

6.2 Limitations and Actions

6.2.1 If the sampling organization has its own sampling procedure, sample custody procedure, labels, or custody forms, they may be substituted for the contents of this procedure as permitted by the sampling plan.

6.2.2 The number of persons handling samples from the time of sampling to receipt by the laboratory should be held to a minimum.

6.2.3 Sample containers shall be labeled by attaching tie-on tags, adhesive labels, or by writing on sample containers with indelible markers. Sample containers shall be labeled with sufficient information that they may be traced to sample collection logs, field sheets, or custody records. Choice of adhesive labels or indelible ink should take into consideration that samples may come into contact with melted ice or condensed moisture during shipment or storage.

6.2.4 Individual samples shall be sealed or sample shipping containers shall be sealed with a tamper-proof seal when they will be relinquished by TVA to a common carrier or if the sampling plan requires it. If the samples will remain in the custody of TVA employees from the time of sampling through transport to the laboratory or under lock and key (as in a locked vehicle or storage container) during this time, use of seals is not required. However, even if seals are not required, their use is strongly urged on shipping containers if the sample is to change hands several times in transport.

6.3 Requirements

6.3.1 Apparatus/Equipment

This procedure specifies no additional apparatus or equipment in addition to any sampling plan.

6.3.2 Materials

6.3.2.1 Sample containers specified in the sampling plan shall be utilized.

6.3.2.2 Labels - Samples labels shall have an adhesive which does not readily release when containers become damp.

6.3.2.3 Custody Forms - Sample chain of custody forms shall be used to record custody of samples after sampling from relinquishment by the sampling organization through transport to receipt by the laboratory. The following information shall be supplied on the custody form:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Requested analysis
g. Sampling location
h. Comments
i. Signature of sample collector.

In addition the form shall contain an area so that each relinquishment and receipt of samples may be documented.

Example custody forms are attached as appendices 10.1 and 10.2. Other forms specific to a given project may be developed as long as they contain the minimum information specified above.

Note: If sample collection time and location are already recorded on a field sheet or sampling log, that information need not be repeated on this form provided a copy of the sampling information is transmitted to the laboratory with the custody sheet.

6.3.2.4 Tamper-evident seals - These seals shall be individually numbered or otherwise marked so that they could not be removed and replaced without it being detected. Two styles have been useful for samples or sample containers.

6.3.2.4.1 Adhesive seals advertised as meeting forensic science requirements, such as Kapak brand seals.

6.3.2.4.2 Padlock-style plastic seals for hasps.

6.3.2.5 Field Logbooks or Field Sheets - Sampling activities may be documented in field logbooks or field sheets designed for that purpose. When these are used, they shall contain:

a. Project identification
b. Sample collection date
c. Sample identification
d. Collection time
e. Number of containers per sample identification code
f. Reference to the sampling procedure
g. Sampling location
h. Comments
i. Signature of sample collector.

7.0 PROCEDURE

7.1 Field Operations

7.1.1 Prior to sampling, label sample containers with an adhesive label or with indelible marker. (Note: If the sampling conditions require it, labels may be affixed after sampling and cleaning the outside of the container.)

7.1.2 Document sample information in a field log, field sheet, or the custody sheet if the first two are not provided.

7.1.3 Seal the sample container with an adhesive seal if the sampling plan requires it.

7.1.4 Complete a "Sample Chain of Custody" form.

7.1.4.1 If field logs or field sheets contain collection time and location, these items may be omitted from the form. In that case, draw a diagonal line in that column and attach a copy of the field logs or sheet so that the laboratory may have pertinent sampling information.

7.1.4.2 If a numbered seal is to be used on the shipping container, note that number in the comments section of the custody form.

7.1.4.3 If the shipping container is to be sealed, sign and date the "relinquished" area of the form.

7.1.5 Place the original copy of the paperwork in a plastic bag inside the shipping container. Retain one copy for field files. Transmit a third copy by separate courier, mail or fax to the laboratory.

7.1.6 Place the samples in a shipping container. As required by the sampling plan, place ice (or commercial substitute) and a temperature test bottle in the container as well. Seal the shipping container if the sampling plan requires it. See also 6.2.4.

7.1.7 Deliver the container to be transported to the laboratory.

7.2 Laboratory Receipt (Reference also GLP-0016)

7.2.1 Inspect the seals. Open the shipping container. Inspect the sample custody form to ensure that it is correctly completed. Sign as receiver. Compare the shipping container contents to the information on the form.

7.2.2 If the "relinquished" blank is not completed and the person delivering the samples is present, have that person sign the "relinquished by." Otherwise write "Not completed", date and initial. If a person signs "relinquished by," provide that person a copy of the paperwork.

7.2.2 As required by the sampling plan, measure the temperature of any samples or temperature blanks and record that information on the custody sheet.

7.2.3 Communicate any errors, broken seals, missing seals, broken samples, differing identification numbers, extra samples, missing samples or misidentification to field personnel. Document all discussions by memorandum or database sample comment file. Document all problems and their resolution by memorandum or database sample comment file. If seals show signs of tampering, bring this to the attention of the group leader or team leader.

7.2.4 Refer to GLP-0016 for further sample receipt and log-in instructions.

7.2.6 Following logging, store the samples in a locked, refrigerated storage area as required by the sampling plan or project plan.

7.3 Laboratory Custody

7.3.1 Samples in locked storage areas, being prepared, being processed, or in autosampler trays are considered to be in the custody of the laboratory. When sampling plans require it, laboratory work areas shall be locked when unattended.

7.4 Sample Disposal

7.4.1 When customers request it, samples shall be returned to them following analysis.

7.4.2 Otherwise, dispose of samples after the time period specified in the sampling plan or project plan. If these do not specify a date, samples should be kept no longer than three months after all analyses are complete.

7.4.3 If the sampling plan requires it, document sample disposal in the workorder file, or custody records.

8.0 <u>SAFETY</u>

8.1 Wear rubber gloves and protective eyewear when handling samples unless it is known that the samples are innocuous.

8.2 Avoid contact with samples. Be aware of broken containers, corrosives, irritants, biohazards, flammability, pyrophoricity, reactivity, radioactivity

and toxicity. Inspect labels and shipping information for warnings. When hazards are known, label samples with hazard information if that is not already provided by the customer.

8.3 In case of skin contact, wash thoroughly with soap and water.

8.4 In case of eye contact, hold the eyes open and wash for at least 15 minutes in an eyewash. Call for help.

8.5 Flammable liquids must be refrigerated only in explosion-proof refrigerators to avoid the risk of explosion caused by sparks in the electrical contacts of the compressor.

8.6 In handling samples, be aware of spills on outside of containers. Clean the exterior of containers as needed.

9.0 <u>NOTES</u>

None

10.0 ATTACHMENTS AND APPENDICES

10.1 Chain of Custody Record - TVA 29203 B (RC-CTR 4-94)

CHAIN OF CUSTODY RECORD

Environmental Site Remediation • TVA Environmental Research Center • Muscle Shoals, Alabama 35660

SWMU NO.	SWMU SEQUENCE	SWMU NAME	SWMU LOCATION	PROJECT

SAMPLERS (SIGNATURE)

SAMPLE NO.	DATE	TIME	COMP/GRAB (C/G)	SAMPLE LOCATION	NO. OF CONTAINERS	ANALYTES										REMARKS

RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME	RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY (SIGNATURE)	DATE/TIME
RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY: (SIGNATURE)	DATE/TIME	RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED BY (SIGNATURE)	DATE/TIME
RELINQUISHED BY: (SIGNATURE)	DATE/TIME	RECEIVED FOR LABORATORY BY: (SIGNATURE)		DATE/TIME	LABORATORY (NAME, CITY, STATE)		

TVA 29203 B (RG-CTR 4-94) •COPY 1 - FIELD INVESTIGATOR •COPY 2 - FIELD INVESTIGATOR •COPY 3 - RETAINED BY LABORATORY

"Sample Chain of Custody"

10.2 Sample custody form - General

Sample Chain of Custody
Tennessee Valley Authority
Environmental Applilations CTR-1K Muscle Shoals, AL

Project ____________________ Date of Collection ____________________

	Sample ID	Collection Time*	Number of Containers	Analyses Requested	Location*	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

	Signatures	Date and Time
Collector		
Relinquishing		
Receiving		

* These columns need not be comleted if field sampling sheets containing the same information are attached.

END OF PROCEDURE